



Durian Seed-Derived *nata de durio*: A Novel Potential Dietary Fiber for Intestinal Health and Constipation

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ABSTRACT

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This study explored the health benefits of nata, a bacterial cellulose and potential dietary fiber source derived from durian seeds fermented by *Acetobacter xylinum*, namely *nata de durio* (NDD). Twenty-five male BALB/C mice were divided into five groups. Three groups received varying doses of NDD: 0.02 g/g BW, 0.04 g/g BW, and 0.08 g/g BW, respectively. One group received 0.04 g/g BW of inulin as a positive control (PC), and one group received only water as a negative control (NC). All groups received standard rodent chow and water *ad libitum*. During the 5-week intervention, body weight, defecation frequency, fecal quantity, fecal weight, and fecal consistency were recorded daily. Post-experiment, the mice were euthanized, and their colons were harvested for histological examination. All groups, except the PC group, maintained a healthy weight. The NDD group, especially at a dose of 0.08 g/g BW, showed significantly higher defecation frequency, fecal quantity, and fecal weight than the NC and PC groups, with softer but non-diarrheal feces. The NDD 0.08 group exhibited the best colon histology with the lowest erosion and inflammation scores. This study suggests that NDD supplementation is non-toxic, beneficial for intestinal health, and potentially serves as an anti-constipation agent.

Keywords: Durian seed, *nata de durio*, bacterial cellulose, dietary fiber, intestinal health, constipation

Introduction

Durian (*Durio zibethinus*), colloquially known as the king of fruit, is a tropical fruit indigenous to the Malay Archipelago. The primary producers of this fruit are Thailand, Malaysia, and Indonesia. Characterized by its round, spiny exterior, durian boasts a unique taste, custard-like texture, and a potent aroma. In Indonesia, the durian season spans from September to February, contributing to the country's national fruit production with an annual yield of 1.3 – 1.5 million tons.¹ Presently, the utilization of durian is predominantly confined to its flesh, which is consumed either fresh or as a component in processed foods. Approximately 67-70% of durian production results in waste, primarily in the form of seeds and shells, with minimal efforts directed toward repurposing these by-products.²

Despite the limited initiatives to optimize the use of durian seeds, existing studies revealed their potential for diverse applications. Fresh durian seeds comprise 54.90% moisture, 3.4% protein, 1.58% ash, 1.32% fat, and 18.92% carbohydrates.³ Another study reported a carbohydrate content of 43.6% in whole durian seeds.⁴ Upon flouring, the seeds contain 6.5% water, 6.0% protein, 3.1% ash, 0.4% fat, 10.1% crude fiber, and 73.9% carbohydrates.⁵ Given their high carbohydrate content, durian seed flour could serve as a substrate for producing nata, a cellulose gel formed by the fermentation of *Acetobacter xylinum*. Currently, coconut water is the primary source of glucose used to make nata, so it is very familiar as *nata de coco*.⁶

Fiber is a food component that is often overlooked. Dietary fiber is part of the plant food source material that is resistant to enzymatic digestion. It encompasses cellulose, hemicellulose, pectin, gums, mucilage, and lignin.⁷ Global dietary fiber intake is generally below the WHO-recommended daily intake of 25-30 grams.⁸ In Indonesia, with a population of 273 million, a staggering 95.5% do not meet the recommended dietary fiber intake.⁹ Numerous studies affirm the extensive health benefits of adequate dietary fiber consumption, including improved laxation, reduced transit time, modulation of intestinal microbiota, enhanced gastrointestinal mineral absorption, decreased energy intake via increased satiety, lowered blood glucose levels, total and/or LDL cholesterol, and blood pressure.⁸

Nata, a form of bacterial cellulose (e.g., *Acetobacter sp.*), could serve as an alternative dietary fiber source. Nata exhibits several advantages over plant cellulose, including purity, low toxicity, high hydrophilicity, and minimal immune response stimulation. With special treatment, nata can have antibacterial effects, be a hemostatic agent, and help cell attachment and tissue regeneration.¹⁰ Its jelly-like, translucent, and chewy characteristics make nata an appealing dietary fiber source for all age groups. Producing nata from alternative base ingredients, particularly fruit by-products like seeds, is promising and beneficial.

This study presented a novel exploration into the production of nata from durian seeds, a previously underutilized resource. It also investigated the impact of this *nata de durio* on various intestinal health parameters, including body weight, body mass index, stool characteristics, defecation frequency, and colon histology, using a male BALB/C mice model. This innovative approach opens up new possibilities for the utilization of durian seeds and contributes to our understanding of the potential health benefits of *nata de durio*.

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Materials and Methods

Preparation of Nata de Durio

The durian seeds used in this study were sourced from the Sawerigading variety of durian fruit, native to Latuppa Village, Mungkajang District, Palopo City, South Sulawesi, Indonesia (-3.049429131074089, 120.12448577780727) and collected during January 2022. Durian samples were identified at the Biotechnology Integrated Laboratory, Faculty of Animal Husbandry, Universitas Hasanuddin, with test result certificate number 020/T/LBTK-UH/I/2022. The stages of *nata de durio* preparations took place at the Laboratory of Biocellulose Products Development, Institute for Research and Community Service, Universitas Hasanuddin. The selected seeds were thoroughly washed and then boiled for 5 minutes to remove sap and soften the texture. Following this, the seed coats were peeled and then thinly sliced. The slices were then soaked in salt water for 5-10 minutes, occasionally stirring until the mucus was no longer present. These slices were then sun-dried until completely dry.

Dried durian seed slices, weighing 250 grams, were ground using a blender, to which 600 mL of distilled water was added. This mixture was subsequently filtered to remove any residual solids. To this filtrate, 60 grams of table sugar (a carbon source) and 12 mL of mung bean sprout solution (a nitrogen source) were added. The mung bean sprout solution was prepared by mashing 25 grams of mung bean sprouts and dissolving them in 50 mL of water. Additionally, 25 mL of cooking vinegar (acetic acid) was slowly added until the mixture reached the desired optimum pH of 4, after which it was cooked.

Following boiling, 200 mL of the suspension was transferred into a heat-resistant glass container and sealed tightly. Once cooled, 250 mL of a starter culture containing *Acetobacter xylinum* was introduced to the suspension.

The starter culture was prepared using a pineapple weighing approximately 1 kg. The flesh of the pineapple was extracted, mashed with a blender, filtered, and the pulp was collected. Three hundred grams of pineapple pulp was combined with 150 grams of table sugar and dissolved in 150 mL of distilled water. This mixture was then incubated for 14 days at room temperature.

The combined durian seed suspension and starter culture were incubated for 7 days at room temperature, away from direct sunlight, to allow the fermentation process. After 7 days, the white layer that formed on top of the mixture was removed. A total of 100 mL of the suspension was transferred to another sterile container, and the incubation was continued for an additional 7 days until the desired nata layer was formed.

Administration of Nata de Durio to Mice

This research uses BALB/C mice as experimental animals with a protocol that has received ethical approval from the Animal Ethics Committee of the Faculty of Medicine Ethics Commission, Universitas Hasanuddin with letter number 835/UN4.6.4.5.31/PP36/2021. The stages of animal study were conducted at the Animal Laboratory, Faculty of Medicine, Universitas Hasanuddin. The nata, once formed, was separated from the fermentation medium, washed with sterile distilled water, blended until smooth, and then oven-dried to reduce the water content. The dried nata was weighed according to the treatment dose, namely 0.02 g/g BW, 0.04 g/g BW, and 0.08 g/g BW, and dissolved in 100 μ L of distilled water. It was then administered orally using gavage once a day for 5 weeks. The positive control group received inulin at a dose of 0.04 g/g BW, dissolved in 100 μ L of water per day, while the negative control group received 100 μ L of distilled water. Throughout the 5-week experiment, the test animals had free access to standard rodent food and drinking water. The body weight of the mice was monitored weekly.

Assessment of Defecation Patterns

Throughout the 5-week experiment, the frequency of defecations and number of feces, as well as the total weight of feces excreted over 24 hours, were recorded daily. Stools consistency was determined based on Bristol Stool Form Scale (BSFS) where type 1 represents separate hard lumps, like nuts; type 2 represents sausage-shape, but lumpy; type 3 likes sausage but with cracks on its surface; type 4 likes an Italian

sausage, smooth and soft; type 5 represents soft blobs with clear cut edges (passed easily); type 6 represents fluffy pieces with ragged edges, a mushy stool; type 7 represents watery, no solid pieces.¹¹

Histology of Colon

After 5 weeks of treatment, the mice were euthanized, and their colons were removed for histological examination using hematoxylin and eosin staining. The colon histopathology was examined by an expert pathologist and performed blinded, without knowing what treatments were given to each tissue preparation, determined based on a scoring system according to protocols from Neumann M *et al.*¹²: "0" for no erosion; "1" for erosion < 1/2 mucosal thickness + damage area < 25%; "2" for erosion < 1/2 mucosal thickness + damage area < 50%; "3" for erosion > 1/2 mucosal thickness + damage area < 50%; and "4" for erosion > 1/2 mucosal thickness + damage area > 50%. Inflammation was scored as follows: "0" for no inflammation, "1" for mild inflammation, "2" for moderate inflammation, and "3" for severe inflammation.¹²

Statistical Analysis

Data from statistical tests are presented as the mean \pm standard deviation (SD). The test was a one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) test. The statistical tests were conducted using Microsoft Excel and an online calculator available at <https://www.socscistatistics.com/tests/anova/default2.aspx>.

Results and Discussion

Nata de durio: increasing the added value of fruit waste as dietary fiber. Approximately 25-30% of fruits and vegetables processed on an industrial scale end up as waste in the form of peels, seeds, cores, pods, vines, shells, pomace, etc.^{13,14} This estimate does not include fruit and vegetable waste originating from households. The utilization of fruit waste is still in its early stages, and there is considerable room for improvement. However, research indicates that the potential of fruit waste is quite promising. It can be utilized in various industries to produce valuable products, thereby supporting the zero-waste movement and sustainable by-product processing. Fruit waste can serve as a rich source of secondary metabolites, phytochemicals, dietary fibers, and polysaccharides, which can be used to produce pharmacological, nutraceutical, and bioactive resources.^{13,14} One way to enhance the value of fruit waste is by processing it into nata. Fruit wastes are still rich in various carbohydrates, including polysaccharides, sucrose, glucose, and fructose, all of which can be used as raw materials for making nata. In addition to being an alternative source of dietary fiber, nata, with its unique characteristics, can be further processed into various products applicable in many fields. These include the food industry, biomedical, cosmetics, papermaking, textiles, electrical and electronic, water purification, and various other applications.^{13,14}

In this research, nata was made from the carbohydrate content of durian seeds. The successfully formed nata was tested for its benefits as a dietary fiber by evaluating its effect on intestinal health. Nata, which is bacterial biocellulose (BC), is a type of insoluble fiber. It is non-viscous, so it does not form a gel-like substance. It can be partially fermented by intestinal microbiota more quickly than viscous fibers such as pectin, beta-glucans, and guar gum.¹³ The formula for creating *nata de durio* in this research was derived from the preliminary results of our study,¹⁵ which demonstrated that the thickness of *nata de durio* is highest (\pm 0.6 cm) when using 250 grams of dry weight of durian seeds (Figure 1). The crude fiber content in this preparation is 1.48%, which is higher than that of *nata de coco* (0.92%). Test results indicate that the fiber content of *nata de durio* consists of 0.81% cellulose, 0.75% hemicellulose, and 0.78% lignin.

Effect of Nata de Durio toward General Condition and Body Weight of Mice During the Experiment

The mice remained healthy and active throughout the experimental period and consumed the provided chows and water *ad libitum*. Mice in the negative control group and all treatment groups experienced an increase in body weight, as depicted in Figure 2. However, the positive

control group, receiving 0.04 g/g BW of inulin, experienced a decrease in weight in the second week, which then remained constant until the end of the experiment. The change in body weight of NDD 0.08 mice was significantly lower (ANOVA + Tukey's HSD $p=0.023$) than that of the NC group. However, at the end of the experiment, there was no significant difference in the body mass index among mice from all groups.

There is a distinct difference in the effects of insoluble and soluble fiber on changes in body weight.^{16,17} Although this study did not induce an increase in the body weight of mice with modified calorie diets, there were observable differences in the outcomes of the mice's body weight. Mice that received *nata de durio*, which is an insoluble fiber, exhibited weight gain after 5 weeks of experimentation. However, the increase was less pronounced than that of the negative control group. There is limited data regarding the effect of cellulose, especially bacterial cellulose, on body weight. Unlike soluble fiber, which affects the absorption of dietary fat, cellulose does not contribute to food absorption. Instead, it aids in pushing food through the digestive tract and supports regular bowel movements. Therefore, the consumption of *nata de durio* does not appear to affect body weight significantly.^{18,19} Conversely, mice in the inulin group experienced weight loss. This result can be explained by several studies which suggest that inulin consumption can accelerate weight loss through the role of intestinal microbiota and metabolites, as well as by increasing the expression of proteins that enhance glucose and lipid metabolism, such as angiotensin-like protein 4 (ANGPTL4).²⁰⁻²²



Figure 1: Representation of the formation of *nata de durio* starting from durian seeds to the formation of nata. The black line on the side of the bottle shows the thickness of the nata formed (± 0.6 cm).

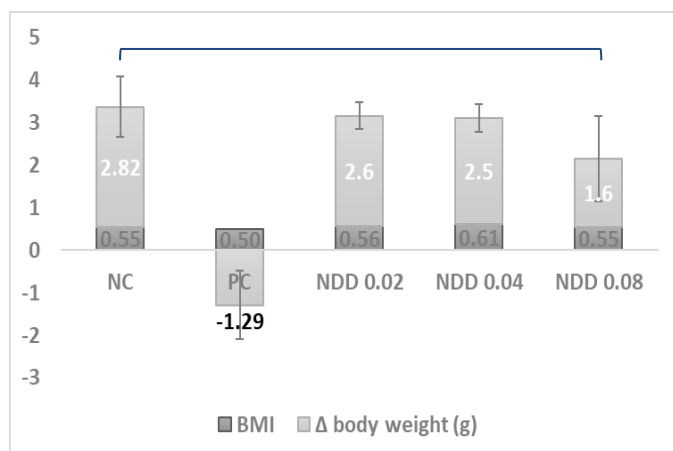


Figure 2: Body mass index (BMI) and changes (Δ) in body weight (g) of mice before and after 5 weeks of experimentation. NC: negative control (distilled water); PC: positive control (inulin); NDD: *nata de durio* 0.02 g/g BW, 0.04 g/g BW, 0.08 g/g BW.

Effect of *Nata de Durio* toward Frequency of Defecation, Quantity, Weight, and Consistency of Feces

During the 5-week experiment, the frequency of defecation per 24 hours in the NDD 0.08 (16.15 ± 1.861) was significantly higher (ANOVA + Tukey's HSD $p < 0.001$) compared to the NC (12.18 ± 1.738) and PC (10.88 ± 1.620) groups, as shown in Figure 3A. The defecation frequencies of NDD 0.02 and NDD 0.04 were also significantly higher (ANOVA + Tukey's HSD $p < 0.001$) than the PC group, with respective values of 13.93 ± 1.803 and 13.20 ± 2.151 but were not significantly different compared to the NC group. The number of feces per 24 hours showed that the NDD 0.02 (73.68 ± 10.383), NDD 0.04 (65.20 ± 6.505), and NDD 0.08 (81.90 ± 11.522) groups were significantly higher (ANOVA + Tukey's HSD $p < 0.001$) than the PC group (59.03 ± 11.479) but were not significantly different from the NC group (68.00 ± 8.351), except for the NDD 0.08 group. Regarding feces weight per 24 hours (Figure 3B), the NDD 0.02 (1.64 ± 0.236), NDD 0.04 (1.757 ± 0.226), and NDD 0.08 (1.87 ± 0.285) groups were significantly higher (ANOVA + Tukey's HSD $p < 0.001$) compared to the NC (1.48 ± 0.250) and PC (1.375 ± 0.306) groups. The feces weights of the NC and PC groups were not significantly different (ANOVA + Tukey's HSD $p = 0.368$).

Recent studies have demonstrated that cellulose has several beneficial functions for gut health. Cellulose plays a crucial role in promoting regular bowel movements and preventing constipation.^{23,24} In this study, the mice that received supplementation with *nata de durio*, particularly at the highest concentration, exhibited the highest frequency of defecation and the most significant number and weight of feces compared to other groups, including the negative control group and those that received inulin supplementation.

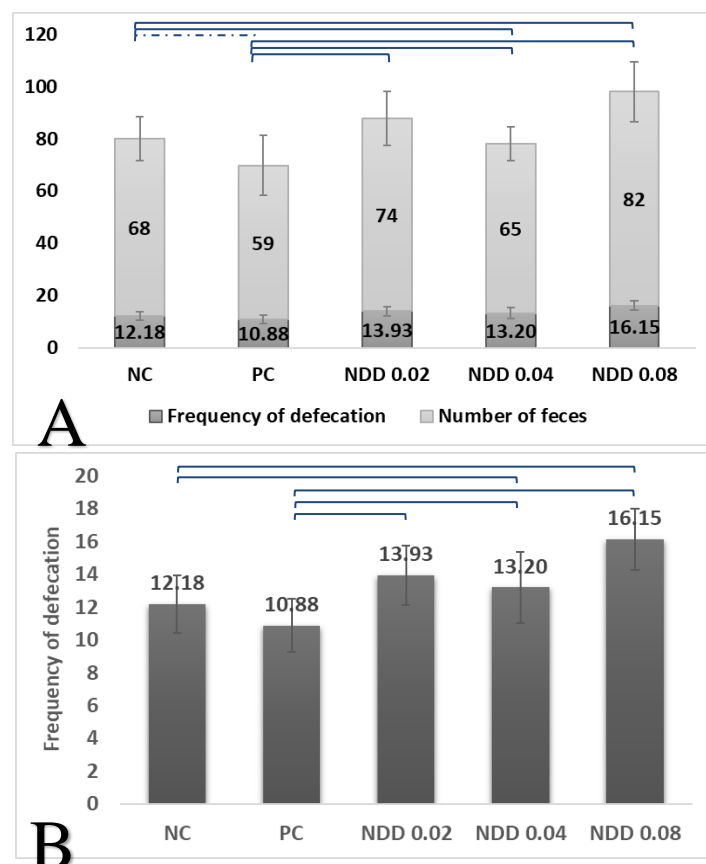


Figure 3: Mean frequency of defecation and number of feces in 24 hours (A), and feces weight in 24 hours (B) during the 5 weeks of the experiment. The lines above the bar charts indicate significant differences between groups. The solid line indicates $p < 0.001$ while the dotted line indicates $p < 0.05$.

As an indigestible material, cellulose resides in the gastrointestinal tract, absorbing fluid and adhering to other byproducts of digestion that are ready to be formed into the stool. Its presence accelerates the movement and processing of waste.¹⁷ Nata exhibits high porosity and hydrophilicity.²⁵ The high density of hydroxyl groups on the surface of BC contributes to its hydrophilic nature. Meanwhile, the formation of extensive H₂ bonds between the chains and the crystalline structure strengthens hydrophobic interactions. These characteristics contribute to BC's amphiphilicity, which can bind various waste substances to form a softer, bulkier, and heavier stool texture, leading to more frequent defecation.^{26,27}

Assessment with BSFS showed that all test groups have an average consistency score in the normal range (Figure 4), namely a score of 3 - 5, where a score of 1 - 2 is in the hard feces category, and 6 - 7 indicates liquid feces such as diarrhea (Table 1). However, there was a significant deviation from this normal range where the NDD 0.04 and NDD 0.08 groups had the highest average score (4.6), and the negative control group experienced the lowest (2.8). Referring to the scale, the feces excreted by the *nata de durio* mice group were, on average, types 4 and 5. The BSFS is widely used to determine whether a stool is normal or abnormal based on its shape and consistency.¹¹ The scale classifies feces into seven types, ranging from separate hard lumps to watery ones with no solid pieces. The optimal types of stools are Type 3 or Type 4, which are sausage-shaped with cracks on the surface and thinner and more snakelike, plus smooth, and soft, respectively. Type 5 stools are soft blobs with clear-cut edges that can pass easily. This type can be considered as a borderline type due to different interpretations; some consider this type to be typical in those without bowel issues, while others interpret it as too loose and may imply diarrhea. However, due to the hydrophilicity effect and the ability of nata to adhere to other materials to form feces, the type 5 feces produced by mice in this study can be considered a normal output. This evidence also demonstrated the potential benefits of *nata de durio* as an anti-constipation agent.

Effect of Nata de Durio on the Colon Histology

The administration of *nata de durio* did not have a toxic effect on the colon tissue of mice. It even provided improvements, as shown in Figure 5, in line with the scoring of erosion and inflammation. The most favorable results were shown by the NDD 0.08 group, which had the minimum erosion and inflammation scores, while the other groups contained mice that experienced mild to moderate erosion and/or inflammation, including the negative control group (Table 2). Histological examination of the colon in this study revealed that *nata de durio*, at a dose of 0.08 g/g BW, resulted in the most favorable colonic appearance. Although this study utilized healthy mice without induced intestinal inflammation, the histology of the mouse colon exhibited erosion and mild to moderate inflammation across all groups. However, the *nata de durio* 0.08 group displayed the least of these conditions. Previous studies have found that dietary cellulose has the potential to induce cellular and molecular anti-inflammatory mechanisms via the maturation of the intestinal microbiota.^{28,29}

For instance, barley leaf insoluble dietary fiber, which includes cellulose, exerts anti-inflammatory effects by modulating the composition of the intestinal microbiota and increasing the production of microbiota-derived metabolites.³⁰ Gut microbiota plays a crucial role in sustaining colonic integrity, partly through producing short-chain fatty acids.³¹ Another study demonstrated that a mixture of insoluble bacterial cellulose and soluble fiber positively affects the modulation of the intestinal microecosystem in mice.³²



Figure 4: Representation of stools excreted by experimental mice during the experiment. A, B, C, and D1, respectively, show stool types 2, 3, 4, and 5, while D2 shows a closer appearance of stools type 5 excreted by mice from the NDD 0.08 group.

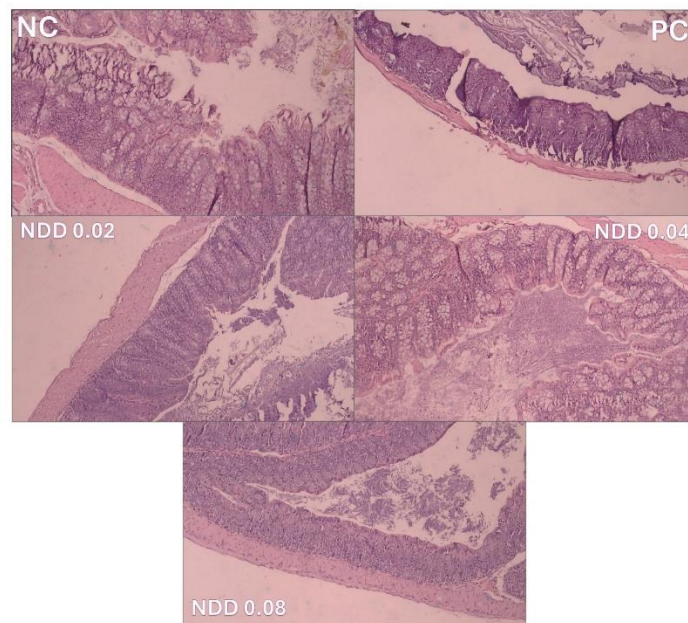


Figure 5: Representative histology of the mice colons with hematoxylin and eosin staining from the NC, PC, NDD 0.02, NDD 0.04, and NDD 0.08 groups after 5 weeks of experimentation. Images were obtained from the light microscope at 10x magnification.

Table 1: Mean stool consistency score based on Bristol Stool Form Scale during the 5 weeks of the experiment

Groups	Week 1	Week 2	Week 3	Week 4	Week 5
NC	2	3	3	3	3
PC	3	4	4	4	4
NDD 0.02	3	4	4	4	4
NDD 0.04	4	4	5	5	5
NDD 0.08	4	4	5	5	5

Table 2: Scores for the degree of erosion and inflammation from the mice colon histology after the 5 week experiment

Sample	Erosion	Inflammation
NC1	2	2
NC2	1	1
NC4	0	0
NC5	1	1
PC2	0	0
PC3	1	2
PC5	0	0
PC6	1	0
NDD 0.02 1	0	0
NDD 0.02 4	1	0
NDD 0.02 5	2	1
NDD 0.02 6	1	1
NDD 0.04 2	1	2
NDD 0.04 4	0	0
NDD 0.04 5	1	1
NDD 0.04 6	0	0
NDD 0.08 1	0	0
NDD 0.08 3	0	0
NDD 0.08 5	1	1
NDD 0.08 6	0	0

The limitation of this study is that no further evaluation was carried out on the microbiota, metabolites, and intestinal immune system to strengthen the explanation of why *nata de durio* at a dose of 0.08 g/g BW can result in the healthiest colonic histology. Therefore, further studies are needed to unravel the potential for more specific anti-inflammatory effects of bacterial cellulose, especially *nata de durio*.

Conclusion

In conclusion, the utilization of durian seeds as raw material for making nata is a form of enhancing the value of waste, which has the potential to bring many benefits, especially in durian-producing areas. This study proves that *nata de durio* does not have toxic effects on healthy mice. The effect on the digestive tract is an increase in the frequency of defecation, with stools that are bulkier, heavier and have a softer consistency. *Nata de durio* had no adverse effects on the colon, even at the highest dose used in this study, providing a protective effect against erosion and inflammation. Further *in vivo* studies and clinical trials are needed to determine the potential of *nata de durio* as an anti-constipation agent and preventative of intestinal inflammation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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