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Effect of Striped Catfish (*Pangasianodon Hypophthalmus*) Supplementation on Mice (*Mus Musculus*) Colon

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Abstract

Diet affects the microbial structure of the gut and human metabolic functions. Disruption of nutrient sources that produce short-chain fatty acids (SCFA) will cause atrophy and inflammation of the colonic mucous. Striped catfish (Pangasianodon hypophthalmus) increases SCFA production because of its high levels of carbohydrates, protein, and fat content. This experimental study used 50 male mice (Mus musculus) aged 3 months old, weighing 20-30 grams, divided into control group (given standard feed) and treatment group (given mixture of standard feed and striped catfish meat). The mice were given treatment for eight (8) weeks at the Faculty of Medicine and Health Sciences, Lambung Mangkurat University, Indonesia, from May to July 2024 and then sacrificed. Colon biopsies were examined using hematoxylin-eosin staining to determine crypt morphology, number of goblet cells, and number of inflammatory cells at Ulin Hospital Banjarmasin, Indonesia, from July until October 2024. The crypt morphology in the treatment group showed fewer abnormalities (5 out of 25 samples) compared to the control group (11 out of 23 samples) (p=0.041) with a moderate correlation (p=0.295). The number of goblet cells was higher in the treatment group (200.4±54.1) than in the control group (134.5±34.3) (p=0.001) with a strong correlation (p = 0.616). The number of inflammatory cells was lower in the treatment group (9.9±4.4) than in the control group (27.6±7.8) (p=0.001) with a very strong correlation (p=0.838). Thus, striped catfish supplementation reduces morphological abnormalities of the crypts and the number of inflammatory cells, as well as increases the number of goblet cells in the colon of mice.

Keywords: Colonic goblet cell, colonic inflammation cell, colonic crypt morphology, short chain fatty acid, striped catfish

Introduction

Colorectal cancer (CRC) and inflammatory bowel disease/IBD (including ulcerative colitis/UC and Crohn's disease/CD) are diseases that can damage colon function.[1] In 2012, the incidence rate of UC in Indonesia was 0.55 per 100.000 people, and CD was 0.33 per 100.000 people.[2] The highest prevalence of CRC in Borneo was reported in South Borneo, with 89.74% of all CRC cases.[3] The diseases were caused by chronic inflammation that alters the immune system and microbiota. Inflammation of the colon mucosa caused by a decrease in the amount of mucinous substance produced by both goblet and epithelial cells which contact bacteria in large quantities resulted in an excessive immune response (characterized by inflammatory

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cell proliferation) from the host and caused abnormalities in crypt morphology.[4]

Gut microbiota composition is strongly influenced by dietary intake, lifestyle, and host health status.[5] Disruption in the availability of dietary substrates such as carbohydrates, proteins, and fats can impair microbial fermentation processes, leading to reduced production of short-chain fatty acids (SCFAs).[6] SCFA is the primary energy source of colonic epithelial cells, and it regulates intestinal physiology and immune function. This is the result of the fermentation of carbohydrates by bacteria, which are difficult to digest and are not digested in the small intestine.[6]

High sources of carbohydrates, proteins, and fats are found in striped catfish (Pangasianodon hypophthalmus) meat which was asccociated with an increase SCFA production and reduce inflammation of the colon mucous.[7] South Borneo has wetland type geography suitable for swamp fisheries development, including striped catfish.[7] A study by Wibowo et al.[7] on the analysis of the composition of striped catfish in South Kalimantan was shown that it contains 1.96% carbohydrates, 2.09% total fats, 19.86% protein, 0.11% omega 3 fatty acids, 0.15% omega 6 fatty acids, and 0.59% omega 9 fatty acids.[7] This nutritional composition, particularly the protein and amino acid content, is higher compared with striped catfish in Bangladesh.[7]

Studies investigating the effects of striped catfish supplementation on colonic inflammation remain limited, despite the high incidence of colorectal cancer and inflammatory bowel disease reported in regions such as Banjarmasin, South Borneo. Therefore, this study aims to evaluate the effect of striped catfish supplementation on colonic health, as assessed by crypt morphology, goblet cell density, and inflammatory cell infiltration in the colon of mice.

Methods

This is an experimental study with randomized posttest-only control group design. Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Lambung Mangkurat University (No. 045/KEPK-FKIK ULM/EC/IV/2024).

The sample size was determined using the Federer formula, ensuring an error degree of freedom of at least 20 to enhance statistical powe. A total of 50 male albino mice (Mus musculus), aged 3 months and weighing 20–30 grams, were included in the study. Animals were randomly allocated into two groups using simple randomization: a control group receiving standard feed and a treatment group receiving a mixture of standard feed and striped catfish meat. Inclusion criteria consisted of healthy male mice within the specified age and weight range, while exclusion criteria included animals that experienced dropout due to illness or death during the study period.

Wild striped catfish (Pangasius sp.) were obtained from the Negara River originating from the Meratus Mountains, Tabalong Regency, South Borneo. The catfish meat was steamed for 10 minutes, ground, and mixed with standard BR2 pellet feed at a ratio of 1:1. The mixture was then molded and dried in an oven at 150°C until completely dehydratedy. Mice were kept and given treatment for 8 weeks at Faculty of Medicine and Health Sciences, Lambung Mangkurat University from May until July 2024, after which they were sacrificed for inspection. Colon tissues were harvested and processed for histopathological examination. Tissue samples were fixed, embedded, sectioned, and stained using hematoxylin–eosin (H&E) until the entire slide surface was adequately covered. Histological evaluations, including crypt morphology, goblet cell count, and inflammatory cell count, were performed using a light microscope in the anatomical pathology laboratory of Ulin Hospital, Banjarmasin, from July to October 2024.

Data were recorded in tabular form and analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0. The crypt morphology was analyzed using the chi-square test, the number of goblet cell was analyzed

Table 1 Comparison Of Crypt Morphology, Goblet Cell Counts, And Inflammatory Cell Counts between the Control and Treatment Groups

Variable	Control group (n=23)	Treatment group (n=25)	p-value	Correlation coefficient (r)
Goblet cell count	134.5±34.3	200.4±54.1	0.001	0.616
Inflammatory cell count	27.6±7.8	9.9±4.4	0.001	0.838

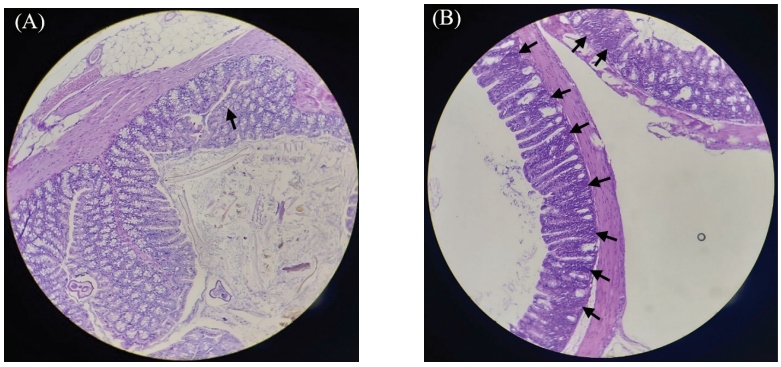

Figure 1 Crypt Morphology in the Control Group Colon Histopathology
(A) Crypt distortion characterized by branching of the crypts (black arrow) (H&E, ×10); (B) Mild crypt distortion accompanied by crypt abscess (black arrow) (H&E, ×100)

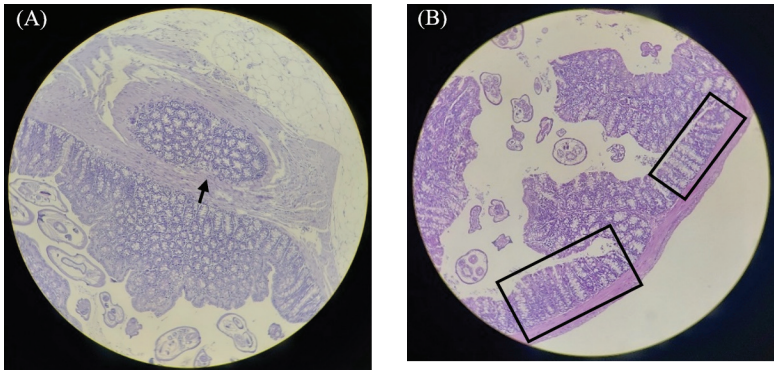

Figure 2 Comparison of Crypt Morphology Between Groups
(A) Control group showing crypt distortion with crypt herniation (arrow) (H&E stain, original magnification ×100); (B) Treatment group showing normal crypt morphology with symmetrical palisade arrangement (square) (H&E stain, original magnification ×100)

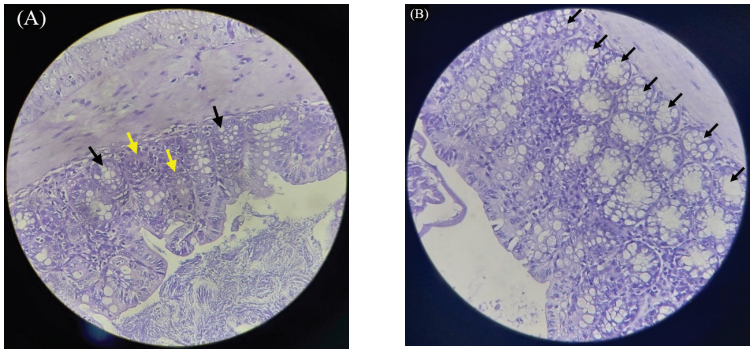

Figure 3 Inflammatory Cell Infiltration in Colonic Tissue
(A) Control group showing increased inflammatory cell infiltration (arrow); (B) Treatment group showing reduced inflammatory cell infiltration (arrow) (H&E stain, original magnification ×400)

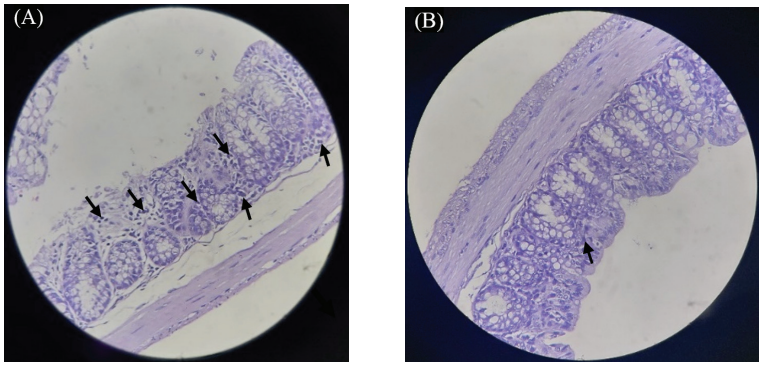

Figure 4 Comparison of Crypt Morphology Between Groups
(A) Control group showing crypt distortion with crypt herniation (arrow) (H&E stain, original magnification ×100); (B) Treatment group showing normal crypt morphology with symmetrical palisade arrangement (square) (H&E stain, original magnification ×100)

using the independent t-test, the number of inflammatory cells was analyzed using Mann–Whitney test. A p-value of less than 0.05 was considered statistically significant. Correlation strength was interpreted as very weak (r=0.00–0.25), moderate (r=0.26–0.50), strong (r=0.51–0.75), very strong (r=0.76–0.99), and perfect (r=1.00).

Results

During the study period, two mice in the control group were excluded from analysis due to non–study-related mortality, while no exclusions occurred in the treatment group. The final analysis included 48 mice, comprising 23 mice in the control group and 25 mice in the treatment group.

During the study period, two mice in the control group were excluded from analysis due to non–study-related mortality, while no exclusions occurred in the treatment group. The final analysis included 48 mice, comprising 23 mice in the control group and 25 mice in the treatment group.

The average number of goblet cells in the treatment group (200.4±54.1) was higher than that in the control group (134.5±34.3) (Figure 3). The number of goblet cell were analyzed using an independent t-test which resulted a significant effect of striped catfish meat supplementation on goblet cell count with p=0.001 with strong correlation (r=0.616).

Abnormalities in crypt morphology were observed in 11 of 23 mice (47.8%) in the control group and in 5 of 25 mice (20.0%) in the treatment group. Statistical analysis demonstrated a significant association between striped catfish meat supplementation and crypt morphology abnormalities (p=0.041; r=0.295). Representative histopathological findings of crypt morphology in the control and treatment groups are shown in Figures 1 and 2, respectively.

Discussion

Striped catfish is among the foods in the Mediterranean diet that affects the gut microbiota and health.[8] The meat contains complete macronutrients consisting of carbohydrates, proteins, and fats.[7] The macronutrients will be further broken down, like protein will be broken down into amino acids, and fat will be broken down into fatty acids which are useful for increasing the variety of gut microbiota.[9] Carbohydrates will be absorbed in the form of glucose and fermented by gut microbiota into SCFAs, namely acetate, butyrate and propionate.[6,8] Previous studies have demonstrated that Mediterranean-style diets increase the abundance of SCFA-producing bacteria, such as *Roseburia*, *Ruminococcus*, and *Faecalibacterium prausnitzii*, which are associated with improved gut health.[8]

Butyrate and propionate functions to increase colon crypt proliferation which directly increases the number of intestinal stem cells. The crypts are the sites of intestinal stem cell (ISC) proliferation and differentiation. Intestinal stem cells are progenitor cells that differentiate into other cells, including goblet cells.[10] The symmetrical division of replicating crypts has been studied in baby mice, starting from the base

to the top and forming two identical crypts.[11] Acetate, butyrate, and propionate also function to increase Tregs cells, including FoxP3$^+$ T cells, which function to express CLTA4, TGF-β1, IL-35, galectin-1, granzymes, and effector molecules to suppress inflammatory cells (lymphocyte cells),[12] which will reduce the occurrence of abnormalities in colon crypts or so-called crypt distortion. Crypt distortion is a picture of crypt damage due to infection and inflammation that lasts for quite some time and is usually found in the chronic phase as well as during the remission phase where inflammation has been reduced by treatment.[11] Crypt distortion is characterized by the presence of branching of the crypt, crypt herniation, crypt abscess, or asymmetrical crypt.

In this study, 11 out of 23 samples in the control group experienced abnormality in the crypt morphology, whereas in the treatment group, 5 out of 25 samples experienced abnormalities. There was a correlation between striped catfish meat supplementation and crypt morphology abnormalities (p=0.041) with moderate correlation (r=0.295).

Dietary composition has been widely recognized as a key regulator of intestinal epithelial signaling and immune modulation. [13] A systematic review reported that western dietary patterns (red meat, processed meat, processed seeds, and high fat dairy products) were significantly associated with an increased risk of colorectal cancer compared with a diet consisting of fruits, vegetables, low fat dairy products, and fish.[13] This is in line with the results obtained from this study, in which mice that were given an extra striped catfish supplementation in their diet showed better results with a decreased number of abnormal crypt morphology in the colon.

This study has similar result with the research by Zhang et al.[14] and Hsu et al.[15] who concluded that SCFA has an immunomodulatory effect that stimulates crypt proliferation, reduces inflammation in the colon of mice, and improves intestinal barrier function, which is also same with research by Ho et al.[13] where SCFA shows an impact on the proliferative capacity of Lgr5+ ISC during homeostasis.

SCFA, especially butyrate also functions in increasing the production of MUC2, which will increase mucus production in goblet cells for intestinal defense system.[4] The intestinal defense system depends on the interaction of barrier components, including the mucus layer, epithelial layer, intercellular tight junction, and lamina propria. The mucus layer formed by goblet cells is the most important thing to maintain intestinal homeostasis.[4] Mucin is hydrophilic and can bind water to form a gel-like structure that prevents direct contact between the mucosa and intraluminal pathogenic microorganisms. A study on mice done by Yang et al.[4] stated that defects in the mucus layer cause bacteria to contact epithelial cells in large numbers, causing an immune response in the host that causes colitis.

In this study, the average number of goblet cells in the treatment group (200.4±54.1) was higher than that in the control group (134.5±34.3). It was concluded that striped catfish meat supplementation had a significant effect on the number of goblet cells (p=0.001) with a strong correlation (r=0.616).

Meanwhile, research conducted by Hsu et al.[15], said that butyrate is an effective modulator of mucin-related gene expression both in vitro and in vivo, and it correlates with the increase in mucus production along with the goblet cell count. The results from that study are also according to research done by Ho et al.[13] that used mice as experimental animals. The study stated that mice lacking dietary fiber for 14 days experienced a decrease in the number of goblet cells, resulting in an increase in yhe number of mucus-damaging bacteria, thinning of the mucosal layer, and colitis. These three sudies are all align with the results of this study.

According to Gallausiaux et al.,[16] SCFA, especially butyrate, plays a role in increasing cell differentiation which increases the number of goblet cells along with MUC2 production in both in vitro and human colon biopsies. Fusco et al.[17] also stated that SCFA, especially butyrate, affects the quality and quantity of goblet cells and mucus production. In a study conducted by Yao et al.[18] using in vitro human cells, it was found that butyrate and propionate stimulate the production of MUC2 by goblet epithelial cells. The aforementioned studies agree with this study result, whereas the mice that received striped fish meat gave better results, with an increased goblet cell count in the colon. These phenomena are likely due to increased mucus production, which protects the mucosa and makes it more difficult for phytogenic bacteria to contact the colon mucosa directly, and reduces the inflammation.

Inflammation in the colon is influenced by IL-22 and IL-10, both of which will limit the development of infection and inflammation.[19] In a study of mice that were given butyrate, it was found that the production of IL-22 and IL-10 in the

colonic lamina propria increased, which caused the number of lymphocyte cells in the lamina propria to decrease. Based on the research by Porbahaie et al.[20] which was conducted in vitro, it was found that butyrate and propionate are the most potent SCFAs to inhibit lymphocyte cell production.

Consistent with these mechanisms, the mean number of inflammatory cells was lower in the treatment group (9.9±4.4) than in the control group (27.6±7.8). This result suggests that striped catfish meat supplementation has a significant effect on the number of inflammatory cells (p=0.001) with a very strong correlation (r=0.838).

Supporting evidence from Yang et al.[19] demonstrated that SCFAs promote $CD4^+$ T cell and IL-22–producing innate lymphoid cell activity, contributing to intestinal homeostasiss. This result is also alike with the research of Akhtar et al.[21] which stated that SCFA plays a role in modulating anti-inflammatory cytokines IL-10 and IL-22 that are important to immune systems as homeostasis mechanisms by limiting inflammatory responses and reducing tissue damage during inflammation in the intestine while maintaining intestinal epithelial cell barriers. According to Ho et al.[13] mice that experienced fiber deficiency for 14 days experienced colitis due to the lack of suppression of pro-inflammatory cytokine production. This is consistent with the results of this study, whereas it is believed to be because SCFA contained in striped catfish meat was asccociated with an increase the production of IL-10 and Il-22 which will limit the inflammatory response and maintain the intestinal epithelial cell barrier.

Several limitations should be considered. First, the use of an animal model may limit direct translation to human physiology. Second, the occurrence of animal dropouts may have introduced bias. Third, SCFA concentrations were not directly measured, limiting mechanistic confirmation of the observed effects.

In conclusion, striped catfish supplementation is associated with improved colonic morphology, enhanced goblet cell density, and reduced inflammatory cell infiltration in mice. These findings suggest that dietary inclusion of striped catfish may support intestinal health and reduce inflammation through microbiota-related mechanisms. Future studies should evaluate different supplementation doses, intervention durations, and direct SCFA measurements to strengthen causal interpretation and clinical relevance.

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