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**Short Communication**

# Comparative analysis of short-chain fatty acid levels in non-alcoholic steatohepatitis rat model: Impact of high-fat high-fructose (HFHF), high fat, and Western diets

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## Abstract

The evidence on the role of diets in the production of short-chain fatty acids (SCFAs) was limited. The aim of this study was to assess the potential effects of high-fat high-fructose (HFHF), high-fat, and Western diets on the levels of SCFA. A research experiment employing a post-test-only control group design was carried out from January to April 2022. A total of 27 rats were randomly allocated to each study group. SCFA was measured two weeks after diet administration. Analysis of variance (ANOVA) test was used to analyze the differences among groups, and the effect estimate of each group was analyzed using post hoc Tukey. The concentrations of SCFAs post HFHF diets were recorded as follows: acetic acid at 54.60±10.58 mmol/g, propionic acid at 28.03±8.81 mmol/g, and butyric acid at 4.23±1.68 mmol/g. Following the high-fat diet, acetic acid measured 61.85±14.25 mmol/gr, propionic acid measured 25.19±5.55 mmol/gr, and butyric acid measured 6.10±2.93 mmol/gr. After the administration of Western diet, the levels of SCFA were 68.18±25.73, 29.69±12.76, and 7.48±5.51 mmol/g for acetic acid, propionic acid, and butyric acid, respectively. The level of butyric acid was significantly lower in HFHF diet group compared to the normal diet (mean difference (MD) 6.34; 95%CI: 0.61, 12.04; $p$=0.026). The levels of acetic acid ($p$=0.419) and propionic acid ($p$=0.316) were not statistically different among diet types (HFHF, high-fat, and Western diet). In conclusion, HFHF diet is associated with a lower level of butyric acid than the normal diet in a rat model.

**Keywords**: Acetic acid, butyric acid, propionic acid, short-chain fatty acid, high-fat high-fructose diets

## Introduction

Short-chain fatty acids (SCFAs) are the main metabolites produced through the anaerobic fermentation of non-digestible polysaccharides by the gut microbiota [1]. SCFAs are classified into formic, acetic, propionic, butyric, valeric, and caproic acids; however, the majority of SCFAs



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that exist in the human colon, almost 90–95%, are acetic acid, propionic acid, and butyric acid [2]. The production of SCFAs is complex, and it may have a close relation with gut microbiota homeostasis. The involvement of SCFAs in the development of intestinal diseases has been reported, such as irritable bowel syndrome, functional constipation, diarrhea, inflammatory bowel diseases, and colorectal malignancy [3]. Moreover, several factors affect the production of SCFAs, for example metabolic disorders, dysregulation of the immune system, psychological stress, and the diets [4].

Diets have been widely known to contribute to the development of some pathological conditions, including intestinal disease [5]. In the development of intestinal diseases, the roles of several SCFAs have been confirmed, such as acetic acid in colitis [6], propionic acid in irritable bowel syndrome [7], and butyric acid in inflammatory bowel diseases [3]. In the context of SCFA production, high fructose diets may be related to heightened production of acetic acid and lower synthesis of butyric acid; high-fat diet is associated with lower production of butyric acid [8]; and sucrose is associated with the formation of acetic, propionic, and butyric acids [9]. To date, information of comparison among high-fat high-fructose (HFHF), high fat (HF), or Western diets on affecting the levels of SCFAs is limited. Therefore, the objective of this study was to compare the impact of HFHF, HF, and Western diets on the levels of SCFAs in animal models. This study might provide preliminary information on which diet has the strongest effect on SCFA levels.

# Methods

## Study design and setting

An experimental investigation utilizing a post-test-only design was carried out at the Biomedical Laboratory of Universitas Brawijaya, Malang, Indonesia, from January to April 2022. A minimum sample size of 28 rats was required and allocated randomly into four study groups with different types of diet: normal, HFHF, HF, and Western diets. The rats underwent adaptation diets for one week, followed by the normal, HFHF, HF, and Western diets for a duration of 12 weeks. Subsequently, the rats were prepared for the examination the levels of SCFAs. The study adhered strictly to Indonesian legal regulations and guidelines governing the utilization of laboratory animals.

## Animal preparation and diets

Male brown rats (*Rattus norvegicus*) at 8–12 weeks of age were purchased from the Physiology Laboratory, Universitas Gadjah Mada, Indonesia and used in this study. The investigation took place in Animal Facility at the Biomedical Laboratory, Universitas Brawijaya, Indonesia. The temperature in the animal room was kept within a range of 23±10°C. The rats were provided with an adaptation diet for one week. Subsequently, the rats were fed with various diets: normal diet (19.2% protein, 67.3% carbohydrate, and 4.3% fat) [10], HFHF diet (24% protein, 41% carbohydrate including 30% fructose, and 24% fat) [10], HF diet (20% protein, 20% carbohydrate, and 60% fat) [11], and Western diet (16% protein, 57.6% carbohydrate, including 34% sucrose, and 16.4% fat) [11].

## Sample preparation

The collection of fecal SCFAs samples was conducted according to the protocols described in a previous study [7]. Briefly, a total of 0.5 g of fecal sample was used and diluted with sterilized water with a ratio of 1:4. The sample was then mixed vigorously for one minute, and the mixture was centrifuged at 4,800 g for 15 minutes at 4°C. The supernatant containing SCFAs was filtered through a cellulose acetate membrane (GyroDisc, Orange Scientific, CA, US) and preserved at -80°C until subsequent analysis.

## Short-chain fatty acids (SCFAs) measurement

The procedure for assessing SCFAs through high-performance liquid chromatography (HPLC) was modified from a prior investigation [7]. In our current study, we assessed three varieties of SCFAs: acetic acid, propionic acid, and butyric acid. Briefly, 40 µl of fecal sample extract was introduced into the HPLC system (Shimadzu LC-10AD Liquid Chromatography, Kyoto, Japan) equipped with a Shimadzu SPD-6A UV-VIS detector (Shimadzu, Kyoto, Japan). SCFAs present

in fecal samples were isolated utilizing an ion exchange resin (Aminex HPX-87H, 300×7.8 mm, Bio-Rad Laboratories, Richmond, US) at a temperature of 65°C. A UV detector set at a wavelength of 210 nm was used to detect the target compounds. A mobile phase consisting of filtered 0.01 N $H_2SO_4$ was utilized, flowing at a rate of 0.6 ml/min.

A method of external calibration standard curves was used to quantify the fecal SCFAs samples. Three calibration standards were employed at six different concentration levels, spanning from 0.01 M to 0.06 M for acetic acid, and from 0.02 M to 0.12 M for both propionic acid and butyric acid. The reference sample was injected into the HPLC system (Shimadzu LC-10AD Liquid Chromatography, Kyoto, Japan) with nine repetitions for measuring the retention time. The calibration curves were assessed by correlating the relative peak area with the molarity of the solution using Open Lab EZ-Chrom software (CA, US). The concentration of fecal SCFAs was presented as the mean μmol per gram wet weight of feces.

### Statistical analysis

Analysis of variance (ANOVA) was utilized to analyze the variance in SCFAs levels among the different dietary groups. The post hoc Tukey test was used to compare groups and ascertain the effect estimates. A significance level of $p<0.05$ was deemed statistically significant. The effect estimate was expressed through the mean difference (MD) along with its corresponding 95% confidence interval (95% CI). Data analysis was conducted utilizing Statistical Product and Service Solution 18 (SPSS 18, SPSS Inc., Chicago, IL, USA). A box and whisker plot was utilized to visually represent the comparison of SCFAs across different groups.

## Results

### Acetic acid level

The levels of acetic acid after the administration of normal, HFHF, HF, and Western diets were: 66.43±7.86, 54.60±10.58, 61.85±14.25, and 68.18±25.73 mMol/g, respectively (**Table 1**). The levels of acetic acid between the diets were not statistically different among HFHF, HF, or Western, and the normal diets.

Table 1. Levels of acetic acid, propionic acid, and butyric acid after given the different type of diets

| SCFAs | Type of diet | | | | *p*-value |
|---|---|---|---|---|---|
| | Normal | High-fat high-fructose | High fat | Western | |
| Acetic acid (mMol/gr) | 66.43±7.86 | 54.60±10.58 | 61.85±14.25 | 68.18±25.73 | 0.419 |
| Propionic acid (mMol/gr) | 21.28±5.03 | 28.03±8.81 | 25.19±5.55 | 29.69±12.76 | 0.316 |
| Butyric acid (mMol/gr) | 10.56±4.14 | 4.23±1.68 | 6.10±2.93 | 7.48±5.51 | 0.038 |

### Propionic acid level

After the administration of the diets, the levels of propionic acid were 21.28±5.03, 28.03±8.81, 25.19±5.55, and 29.69±12.76 mMol/g for normal, HFHF, HF, and Western diets, respectively (**Table 1**). The levels of propionic acid were not significantly different among types of diets.

### Butyric acid level

The concentrations of butyric acid were 10.56±4.14, 4.23±1.68, 6.10±2.93, and 7.48±5.51 mMol/g among animals within normal, HFHF, HF, and Western diet group, respectively (**Table 1**). The level of butyric acid was lower in HFHF diet group compared to the normal diet (MD: 6.34; 95% CI: 0.61, 12.04; $p=0.0260$) (**Figure 1A**). Whereas the levels of butyric acid after the administration of HF diet (**Figure 1B**) and Western diet (**Figure 1C**) were similar to the normal diet.

## Discussion

To the extent of our understanding, our study represents the initial documentation comparing the levels of SCFAs among various types of diets in a rat model. We showed that butyric acid was the only SCFA affected by diet administration, and HFHF was the only diet having an impact on governing the production of butyric acid. Our study was supported by the findings of previous

studies [8,12,13]. HFHF diet had been proven to affect the balance of intestinal microbiota and increase cholesterol levels [8], a condition linked to a heightened susceptibility to intestinal disorders such as ulcerative colitis, Crohn's disease and colon cancer [12,13]. The mechanism of how HFHF diet affected chronic inflammation in the intestinal layers has shown previously [14,15]. Studies also showed that butyric acid was a prominent compound with a pivotal role in the development of intestinal diseases [16-18]. Additional corroborating evidences indicated that individuals with ulcerative colitis and Crohn's disease exhibited lower levels of butyric acid compared to healthy individuals [17,18], suggesting that butyric acid could potentially offer protection against inflammatory bowel disease. In our present study, consumption of the HFHF diet might lead to disturbances in gut microbiota balance, potentially resulting in reduced production of butyric acid, an SCFA synthesized by gut microbiota in the colon [19]. The above explanation suggests that the HFHF diet could induce a reduction in levels of butyric acid, as reported in our study.

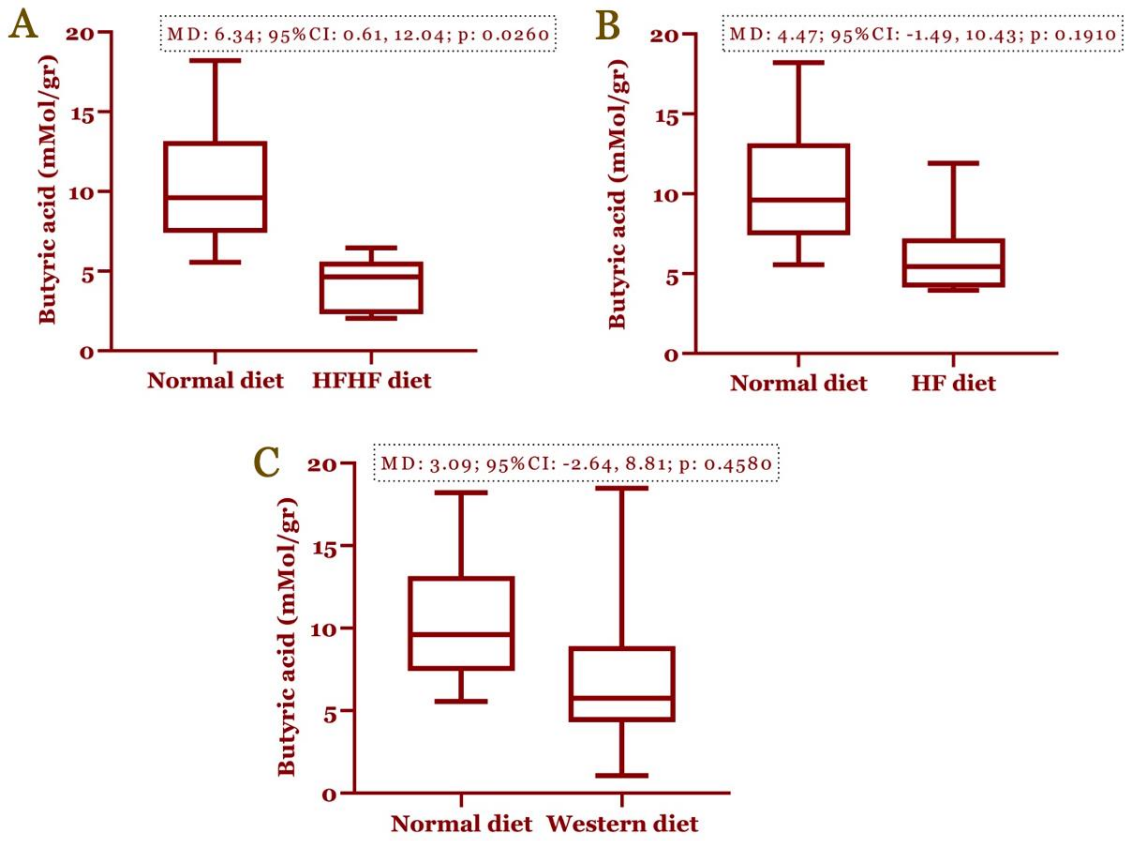


Figure 1. The comparison of different dietary patterns and their impact on butyric acid levels. (A) High-fat high-fructose diet (HFHF) diets vs normal diet. (B) High fat (HF) diet vs normal diet. (C) Western diet vs normal diet.

Our study also found similar levels of acetic acid and propionic acid after HFHF, HF, and Western diets compared to normal diets. These findings might be complicated to describe. In the context of intestinal microbiota homeostasis, the impact of diets on the levels of acetic acid and propionic acid should be in line with the levels of butyric acid. The disruption of the balance in gut microbiota caused by the administration of various diets might contribute to the production of acetic acid and propionic acid [20]. However, previous studies had shown comparable levels of acetic acid and propionic acid between individuals with inflammatory bowel disease and those without the condition [21-23], suggesting that the degree of inflammation in the intestinal layers did not have enough impact to alter the production of acetic acid and propionic acid.

The mechanisms by which the HFHF diet influences butyric acid levels are not thoroughly understood. Nonetheless, certain prior studies suggested a potential mechanism [2,3,8]. Consumption of the HFHF diet might modify the gut microbiota composition by decreasing *Megasphaera elsdenii*, a bacterium classified within the Firmicutes family, known for its capacity

to convert lactates into butyrate [3]. Moreover, decreasing the presence of beneficial *Bifidobacterium* and *Lactobacillus*, the bacteria that have cross-feeding interaction with *Firmicutes* to produce butyric acid, was also reported after HFHF diet [2,8]. Those possible mechanisms may explain how HFHF diet affects the impaired production of butyric acid.

Our present study had some limitations. Potential covariates, such as the levels of cholesterol and gut microbiota, which might be affected by diet administration, were not analyzed. Additionally, because of the limited sample size of animals tested, our current findings should be interpreted with caution. Therefore, further study with a larger sample size with an evaluation of potential confounding factors such as cholesterol levels and gut microbiota should be conducted to achieve more robust findings.

## Conclusion

Our study suggests that HFHF diet administration is associated with lower levels of butyric acid in a non-alcoholic steatohepatitis rat model. The present study might contribute to a better understanding of the potential impact of diets on the levels of SCFA. This finding might also provide potential information on the use of butyric acid as a potential therapeutic in the case of intestinal inflammation. However, the proper mechanism of how HFHF diets affect the production of butyric acid requires further studies.

### Ethics approval

The Ethical Committee of Universitas Brawijaya, Malang, Indonesia (No. 022-KEP-UB-2022) approved all animal protocols in this study. Furthermore, all procedures were conducted in accordance with Indonesian laws and guidelines pertaining to the use of experimental animals.

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### Competing interests

All authors affirm that they have no conflicts of interest.

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### Underlying data

The original data supporting the conclusions of this study can be obtained from the corresponding author upon request.

## How to cite

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