High-fat and high-fructose diet lowers the cecal digesta’s weight and short-chain fatty acid (SCFA) level of Sprague-Dawley rat model

Abstract

Objective: This study aimed to analyze the effect of a high-fat and high-fructose diet (HFFD) on the digesta weight and short-chain fatty acid (SCFA) levels of cecal digesta in rats.

Method: This study was an experimental study with a posttest-only control group design with male Sprague Dawley strain rats as the samples. A total of 36 rats were divided into two groups with normal diet (N) and modified HFFD. The data of energy intake, nutrients and fiber, body weight, Lee index, abdominal circumference, digesta weight, and SCFA levels of cecal digesta were collected.

Results: The results showed an 11.94% increase in body weight of rats with HFFD. The total energy intake of the HFFD group was significantly higher than N (p = 0.000). The fiber intake and cecal digesta weight in group N were higher than that in the HFFD group (p = 0.00 and p = 0.02, respectively). The concentrations of acetate, butyrate, propionate, and total SCFA in N group were significantly higher than in the HFFD (p = 0.041; p = 0.004; p = 0.040; p = 0.013, respectively). A significant negative relationship was observed between the abdominal circumference and cecal digesta concentration (p = 0.029; r = −0.529) and between the Lee index and the SCFA concentration of cecal digesta (p = 0.036; r = −0.206).

Conclusions: The research results showed that HFFD can reduce the weight and SCFA concentration of the cecal digesta. The negative relationship between abdominal circumference,
the Lee index, and the SCFA concentration indicates the potential role in obesity incidence and metabolic diseases.

Keywords: cecaldigesta; high-fat high fructose diet; SCFA

1. Introduction

Obesity is a result of the imbalance between energy spending and the intake that influences various metabolic pathways related to metabolites and hormones[1]. The number of people with obesity has been increase and predicted will be increased up to 57.8% especially for the adult population in the world in 2030 (Shrimpton & Rolox, 2013; Forse & Krishnamurty, 2015). Current studies suggest that obesity often accompanied by diseases such as insulin resistance, dyslipidemia, and hypertension as well as increases the risk of type 2 diabetes and cardiovascular diseases [2] (Lavie, 2012; Malik & Hu, 2012; Landsberg et al., 2013). Based on the data from the Basic Health Research (Riskesdas) in 2018, the prevalence of obesity was increased from 14.8% in 2013 to 21.8% in 2018 in Indonesia [3].

Several studies explained that increased consumption of foods and beverages with high-fructose corn syrup (HFCS) would induce the increase of the obesity prevalence. Fructose can facilitate the glucose absorption but it will lead to mal-absorption when the fructose content is higher than glucose. Fructose has more potential to generate hepatic uric acid and triglycerides which causes fatty liver than sucrose (Lozano et al., 2016). Mamikutty et al. (2014) reported that rat subjects had obesity, hypertension, hyperglycemia, and hypertriglyceridemia after drank water with 20% fructose for 8 weeks [4]. Fructose causes addiction and leptin resistance in the brain while also decreases the expression of cholecystokinin and the growth hormone expression in the ventromedial nucleus. Long-term fructose consumption increases the calorie intake due to the loss of satiety signals in the brain, eventually, resulting in obesity [5-7].
Besides, a low-fiber content in diet is one case that causing low short-chain fatty acid (SCFA) production by the intestinal microflora. SCFAs, such as acetate, propionate, and butyrate are produced through fermentation by intestinal microflora from dietary fibers [5]. One of the roles of the intestinal microflora is to convert the free fatty acids (FFAs) from dietary fats to other FFAs as their metabolism results, which are the key factor in energy metabolism [8, 9]. Dietary fiber has a beneficial metabolic effect on body weight as well as glucose homeostasis, food intake, and insulin sensitivity [8, 9].

SCFA involvement in energy and lipid metabolism has gained attention as the potential in controlling metabolic syndrome. Several studies showed that decreased obesity and insulin resistance occurred in the experimental animals with a high-fat diet after the butyrate-containing food supplementation [6]. The mechanism that explains the higher fecal SCFA production in the obese population remains a subject of debate. It could be due to the increased dietary substrate intake or the consequence of the increased metabolic activity of a certain group of bacteria, interestingly, some studies show inconsistent results. For example, the results of research Fernandes et al. (2014) showed experimental results that are contrary to the results of research conducted by Lin et al, 2012, Weitkunat et al, 2017 and Miyamoto et al, 2018. There was an increase in diet but there was also a decrease in SCFA concentrations.

The controversy over these results prompted researchers to conduct studies on obese rats with high-fat high-fructose diet (HFFD) and measure the weight and concentration of SCFA in the cecum rather than in the feces. Therefore, the present study aims to analyze the effect of HFFD administration on digesta weight and SCFA levels in rat cecum as only 5–10% of SCFA is excreted in the feces [10]. Using the obesity animal model, several factors related to food can be controlled and the concentration of SCFA in the cecum can be analyzed. Thus, this study
provides more objective results that could be used as a basis for creating an experimental animal model of obesity and nutrition interventions. This study has several differences with the previous ones; (1) used Sprague Dawley while some studies used Wistar; (2) used high-fat high fructose for the feed while previous studies used high fat or high carbohydrates; and (3) the SCFA was measured in cecal digesta while others measured it in the feces.

2. Materials and Methods

This research was conducted in October 2017 until February 2018. The ethical clearance for this research was from the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia, No. 368/EC/KEPK/10/2017.

2.1. Materials

The feed was composed of cornstarch, dextrin cornstarch, sucrose, soybean oil, casein, egg white, agar, white butter, beef kidney fat, minerals (AIN-93 M-MX-Mineral Mix), vitamins (AIN-93 -VX-Vitamin Mix), L-cystine, and choline bitartrate.

2.2. Normal and HFFD diet formulation and feed energy and content analysis

The manufacturing of modified feed and analysis of energy content and nutrient of the feed were according to the research by Sulistyowati et. al. (2019) [11]. Feed ingredients consist of cornstarch, dextrin cornstarch, sucrose, soybean oil, casein, egg white, gelatin, white butter, cow kidney fat, minerals (AIN-93 M-MX-Mineral Mix), vitamins (AIN-93 -VX-Vitamin Mix), L-cystine, and choline bitartrate. All ingredients are mixed together, and formed as rat pellets. The composition of dietary diet modification for the intervention in making animal models of obesity in this study are listed in Table 1 [11].

2.3. Animal preparation and experimental
2.3.1. Experimental Design

This study used a posttest-only control group design. The number of replications in this study was determined by the Federer formula [12]:

\[(t - 1)(n - 1) \geq 15 \quad (1)\]

where \(t\) is the number of the intervention group and \(n\) is the number of repetitions or the number of samples per group. The number of interventions was 2 in this study, therefore, the number of replication was at least 16. Then, a number of the backup experimental unit was added to anticipate unwanted possibilities, such as death. The correction to the number of replications was based on the Higgins formula:

\[\frac{1}{(1-f)} \quad (2)\]

with an estimated experimental unit dropout (f) of 10%, the number of subjects needed in this study was 36.

The white rats (\textit{Rattusnorvegicus}) Sprague Dawley strain were obtained from the Animal Laboratory of Institut Pertanian Bogor. The rats were 200–250 g body weight, aged 70–90 days post-natal, male, and healthy. Rats with abnormal motor movements; did not want to eat and drink; and had >10% weight loss during the adaptation period were excluded from the study. Sprague Dawley was chosen as an obese animal model because it is quieter and easier to handle. Besides the fact that the rats can show the changes in metabolic status according to the research objectives, these rats also have a high survival rate [13-14, 20-22].

Rat cages were placed in a ~2-cm-thick husk mat that was replaced every 3 days. Room temperature was maintained at 25°C in 12 hours of dark and light cycles. Rats were acclimated
for 7 days, fed with normal rat feed, and given distilled water for drinking *ad libitum*. On the seventh day, 18 rats in the intervention group were given HFFD and 30% fructose solution drink for 12 weeks [13, 14]. The administration of a normal diet and HFFD was conducted for 12 weeks. Maintenance and euthanasia of rats were done following the fixed procedures at the Institute of Biosciences of Universitas Brawijaya Malang.

2.3.2. Sample collection and preparation

Cecal digesta sample preparation for the SCFA content and digesta weight analysis was carried out by cecal dissections. The contents of the cecum (digesta) were scraped off using tweezers and then weighed using an ABK 220-4M digital scale-Japan. Next, each of the digesta was stored at -20°C for the SCFA examination.

2.4. Determination of parameters

2.4.1. Calorie intake

The feed intake was calculated by subtracting the amount of the given feed (g) and the leftover (g) after 24 hours. The same was applied to the drinks in the HFFD group.

\[
\text{Feed intake} = \text{the provided feed (grams)} - \text{the leftover feed (grams)} \tag{3}
\]

Calorie intake was calculated by the amount of feed intake (g) and 30% fructose solution (mL) multiplied by the energy value of feed N, HFFD, and the 30% fructose solution.

\[
\text{Calorie intake} = \text{feed intake (grams)} \times \text{the energy density of the feed} \tag{4}
\]

\[
\text{Total calorie} = \text{the feed calorie} + \text{the drinks calorie} \tag{5}
\]

2.4.2. Bodyweight, length, abdominal circumference, and Lee index
Bodyweight measurements were carried out by weighing rats with a Kern 440-33N analytical balance (Kern & Sohn, Germany) once a week. The result was the average of the two weighing results. Body length was measured after the rat was anesthetized with ketamine-xylazine of 75-100 mg/kg + 5-10 mg/kg. The body length was measured from the tip of the nose to the anus or base of the tail (naso-anal). Abdominal circumference was also measured after the anesthesia. Abdominal circumference was measured circularly in the largest part of the abdomen. Body length and abdominal girth were measured using OneMed 235 metleen (OneMed, Indonesia) with an accuracy of 0.01 cm. Lee index was used to determine the degree of obesity:

$$\text{Lee index} = \frac{\text{Body weight (g)}^{0.33}}{\text{Body length (mm)}} \times 1000$$  \hspace{1cm} (6)

2.4.3. The weight of the cecaldigesta

The weight of the cecaldigesta was measured by using Kern ABJ 220-4M (Kern & Sohn, Germany) digital scales with an accuracy of 0.000 mg.

2.4.4. SCFA levels

The digest samples stored at -20°C were thawed and 4 mg of the samples were centrifuged at 10,000 rpm for 15 min. As much as 2 mL of supernatant was added into a 5 mL plastic tube and 30 mg of 5-sulphosalicylic acid was added. The solution was shaken, centrifuged at 3000 rpm for 10 min at 4°C, and then filtered through a Millipore filter until the clear liquid was obtained. As much as 1 μL of supernatant was injected into the gas-liquid chromatography device (Chrompack CP 9002 series 946253, Netherlands) using a micro-syringe. After 9 min, the area of the specified compound was drawn in the recorder paper. Before the sample was injected, a standard mixture of acetate, propionate, and butyrate was injected first with a concentration of
0.025%; 0.05%; 0.3%; and 0.5%, respectively. Then the regression equation was calculated, which reflected the relationship between the area of acetic acid, propionic, and butyrate standard (Y) with the concentrations of acetic acid, propionic, and butyrate standard (X) [15-19].

2.6. Data analysis

All observations and measurements were tabulated and made in the form of mean ± standard deviation (SD). The differences in the experimental animals’ characteristics, the weight of the cecum contents, and the concentration of SCFA from both groups were analyzed by using an independent t-test at a 95% confidence level. The correlation between variables was tested by a bivariate test and the relationship was determined using the Pearson’s correlation test. Data processing and analysis were done in Microsoft Excel and SPSS version 21 (IBM) for Windows.

3. Result

The characteristics of the initial subjects and the results of the interventions in this study are presented in Table 1. The rats had a similar bodyweight at the beginning of the study as the mean initial body weight in both groups were not different (p = 0.945). After the HFFD dietary intervention for 12 weeks, there was an increase of 12% in the average body weight, higher than the normal but not statistically significant (p = 0.140). However, there was a significantly higher abdominal circumference in the HFFD compared to the N group (p = 0.009).

The mean digesta weight of rats with N feed was significantly higher than that of rats with HFFD feed (p = 0.02) (Figure 1). Meanwhile, the concentration of SCFA in cecal digesta rat with N feed was significantly higher than in rats with HFFD (Table 2).

Pearson’s correlation test results show a significant negative relationship between the abdominal circumference and SCFA concentration (r = −0.529; p = 0.029). While, the negative relationship
also showed between the Lee Index and the SCFA concentration of the cecaldigesta ($r = -0.204$; $p = 0.433$). The relationship between abdominal circumference, Lee index, and SCFA concentration are presented in Table 3.

4. Discussion

Several studies explained that an increase in the prevalence of central obesity occurs along with the increase in consumption of foods and drinks containing (High Fructose Corn Syrup) HFCS. HFCS is commonly used in a widely favored food and beverage products such as soft drinks, pastries, cookies, gums, jelly, and desserts. In the long run, the addiction effects and leptin resistance due to the fructose will increase the calorie intake because of the loss of satiety signals in the brain. In turn, it will cause overweight and obesity [21].

The intervention of the HFFD feed was recommended in producing experimental animal models of obesity [23]. Previous studies presented that the composition of the HFFD feed is 25%–35% carbohydrate, 50%–70% fat, 15%–25% protein, and 17%–30% fructose solution [13, 14, 20, 24]. In this study, the composition of the HFFD feed was 20.51% carbohydrate, 57.57% fat, and 21.90% protein, with a fiber content of 24.25 g per 100 g of feed, and 30% fructose in drinks.

The results indicated that the modified AIN 93 HFFD did not provide significant changes in body weight. However, there was an 11.94% increase in body weight in the HFFD group. This result is in line with the previous studies that showed that the rat had moderate obesity if its bodyweight change was 10%–25% higher than those in the N group [7, 25]. Those factors did not show a significant increase in the body weight because the modified feed formula still had a higher energy density than the N diet, about 4.21 Cal/g. According to Miras et al. (2014), the average energy density for N feed-in obese rats’ models was <3.5 Cal/g. In this study, the high energy density of N feed was due to the use of carbohydrate source material so it could be easily
molded as a mouse pellet [26]. The calorie intake in this study was not different from the result of Marques et al. (2016), which was around 66 calories for the HFFD group and 51 to 53 calories for the N group [14]. The calorie intake from the feed in the HFFD group was lower than in the N group, however the total calories of HFFD group were significantly higher than N group. The high-calorie intake in the HFFD group was due to the drink that contained 30% fructose. Besides, the HFFD feed had <60% fat was also one of the contributing factors [14].

Fiber intake in the N group was significantly higher than in HFFD. This was due to the lower fiber content in the HFFD feed. The HFFD feed generally has a low carbohydrate content from dietary fiber [8]. It affected the weight of the digesta in the rat cecum. The weight of rat cecal digestion with the N diet was significantly higher than HFFD (p = 0.02). The increasing volume of feces is the effect of the dietary fiber’s metabolism in the digestive tract, especially in colon [27].

Most of the metabolic functions of dietary fiber are related to the colon as the fiber is relatively unchanged in the stomach and small intestine. Bacterial flora works actively in the colon. Besides being used to increase the content and weight/volume of feces, the metabolic products are used to produce volatile fatty acids (acetate, butyrate, and propionate) which are the main anions in feces [27, 28]. The data obtained from individuals who experienced sudden death showed that the SCFA concentrations in the cecum were around 131 mmol/kg lumen contents, ten times higher than those in the ileum (13 mmol/kg) [29, 30].

The results of this study showed that the rats with N feed had significantly higher SCFA concentrations in cecal digesta than the HFFD (p = 0.013). The results showed a high-fiber diet with low fat caused a higher amount of SCFA feces compared with the low-fiber diet [31]. The majority of acetate is produced by enteric bacteria as a result of carbohydrate fermentation.
Besides, one-third of colonic acetate is synthesized from hydrogen and carbon dioxide or formic acid through the Wood–Ljungdahl pathway by acetogenic bacteria [32, 33]. Three different pathways used by colon bacteria for propionate formation are the succinate pathway, the acrylic pathway, and the propanediol pathway [34]. Fermented food fiber with cellulose substrate gives a proportion of 81% of acetate, 13% of propionates and 6% of butyrate. In the fermentation process in the large intestine, cellulose and pectate will produce the largest proportion of acetic acid, while hemicellulose potentially produces propionate acid (Makki et al., 2018). This study showed the amount of produced acetate was higher than propionate and butyrate was the least in quantity.

The results also showed the negative relationship between the increased abdominal circumference, which showed the degree of visceral obesity, with the decreased concentrations of cecaldigesta \((r = -0.529; p = 0.029)\); which was also similar to the Lee Index with the decrease in the SCFA concentration in the cecaldigesta. \((r = -0.204; p = 0.433)\). These were consistent with Heinritz et al. (2016) which explained that SCFA concentrations were significantly higher in the administration of low-fat diets than high-fat; the significance value for acetate, butyrate, and propionate was \(p = 0.023\), \(p = 0.013\), and \(p = 0.003\), respectively. (19)

Similar results were also found in Barczynska et al. (2018) that observed the SCFAs concentration in the feces of obese children was lower than normal-weight children \((p = 0.04)\) [35]. It can be explained that the SCFA protective effect on the metabolic changes induced by a high-fat diet depends on the regulation of proliferator-active peroxisomes (PPAR\(\gamma\)); it triggers the changes of lipid synthesis to lipid oxidation [36]. Interestingly, although the three intestinal SCFAs have a protective effect on obesity, butyrate and propionate are likely to have a more significant effect than acetate [37]. Different mechanisms have been proposed to explain this effect, one of them is the activation of signaling pathways mediated by protein kinases, such as
adenosine monophosphate-activated protein kinases [6, 36] or mitogen-activated protein kinases [38]. It has been reported that butyrate and propionate induce the intestinal hormone production which can reduce the food intake [37]. Acetate also has been proven to reduce appetite through interactions with the central nervous system [39]. One mechanism underlying the effect of SCFA on food intake and satiety is related to the release of intestinal hormone related to satisfaction, especially GLP-1 and the peptide YY (PYY). These proteins are secreted by intestinal L cell enteroendocrine which is found in the distal ileum and colonic epithelium [40, 41]. PYY affects appetite and satiety by suppressing neuropeptide Y (NPY) and activating the proopiomelanocortin (POMC) neurons in the hypothalamus or by delaying the gastric emptying process [42]. In addition to its role as incretin, GLP-1 also regulates the appetite by affecting POMC and NPY neurons in the hypothalamus which also inhibits gastric emptying and gastric acid secretion [43-46]. Unfortunately, the secretion from intestinal hormones (PYY and GLP-1) was not assessed in this study, which became a limitation in this study.

5. Conclusion

The administration of HFFD showed a weight and SCFA concentration reduction in the cecum contents which is harmful to health. However, further experimental study is still needed to get more deep information before it can be applied for human dietary.

Conflict of interest

There is no conflict of interest

References


11. Sulistyowati, E., Handayani, D., Soeharto, S. and Rudijanto, A. Serum mineral (Mg, Mn, and K) levels are associated with increasing the body mass index (BMI) and abdominal circumference. Obesity Med. 209; 15: 100107.


Legends

Table Legends

Table 1: Characteristics of initial subjects and the results of normal and HFFD dietary food intervention

Table 2. Concentrations of SCFA in rat cecaldigesta by group

Table 3. Pearson’s correlation coefficient between waist circumference and SCFA concentration of cecaldigesta and between Lee index and SCFA concentration of cecaldigesta

Figure Legends

Figure 1. The average weight of cecaldigesta by group
Table 1: Characteristics of initial subjects and the results of normal and HFFD dietary food intervention

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal (mean ± SD)</th>
<th>HFFD (mean ± SD)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>246 ± 19.11</td>
<td>245.58 ± 22.06</td>
<td>0.945</td>
</tr>
<tr>
<td>Intake (g)</td>
<td>12.23 ± 1.67</td>
<td>6.49 ± 1.52</td>
<td>0.000</td>
</tr>
<tr>
<td>Intake fiber (g)</td>
<td>0.35 ± 0.05</td>
<td>0.16 ± 0.04</td>
<td>0.000</td>
</tr>
<tr>
<td>Intake (calories from drinks)</td>
<td>0</td>
<td>31.71 ± 7.08</td>
<td>0.000</td>
</tr>
<tr>
<td>Intake (calories from diet)</td>
<td>51.60 ± 7.04</td>
<td>34.59 ± 8.12</td>
<td>0.000</td>
</tr>
<tr>
<td>Total intake (calories)</td>
<td>51.60 ± 7.04</td>
<td>66.30 ± 7.26</td>
<td>0.000</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>261.93 ± 29.30</td>
<td>279.42 ± 33.22</td>
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<tr>
<td>Lee index</td>
<td>265.85 ± 10.15</td>
<td>287.65 ± 10.34</td>
<td>0.875</td>
</tr>
<tr>
<td>Abdominal circumference (cm)</td>
<td>14.64 ± 0.93</td>
<td>15.84 ± 0.41</td>
<td>0.009</td>
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</table>

Source: Sulistyowati et al. (2019)
Table 2. Concentrations of SCFA in rat cecaldigesta by group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>HFFD</th>
<th>p value</th>
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<tbody>
<tr>
<td>Acetate (mMol/g)</td>
<td>22.76 ± 6.68</td>
<td>18.18 ± 6.93</td>
<td>0.041</td>
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<tr>
<td>Butyrate (mMol/g)</td>
<td>3.23 ± 1.54</td>
<td>2.72 ± 1.28</td>
<td>0.004</td>
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<tr>
<td>Propionate (mMol/g)</td>
<td>7.31 ± 2.81</td>
<td>5.26 ± 2.09</td>
<td>0.040</td>
</tr>
<tr>
<td>Total SCFA (mMol/g)</td>
<td>33.30 ± 8.93</td>
<td>26.16 ± 9.94</td>
<td>0.013</td>
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</table>

Table 3. Pearson’s correlation coefficient between waist circumference and SCFA concentration of cecaldigesta and between Lee index and SCFA concentration of cecaldigesta

<table>
<thead>
<tr>
<th></th>
<th>Waist circumference</th>
<th>Lee index</th>
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<tbody>
<tr>
<td>SCFA</td>
<td>-0.529*</td>
<td>-0.204</td>
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*Significant level at $\alpha = 0.05$

Figure 1

<table>
<thead>
<tr>
<th></th>
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<th>HFFD</th>
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<tr>
<td>mean</td>
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<td>1,4289</td>
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