Amerta Nutrition
e-ISSN: 2580-1163 (Online)
p-ISSN: 2580-9776 (Print)
Ilmi et al. | Amerta Nutrition Vol. 8 Issue 3SP (December 2024). 218-227


RESEARCH STUDY
English Version
OPEN ACCESS

# The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome

## *Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik*

**Ibnu Malkan Bakhrul Ilmi[1]*, Angga Hardiansyah[2], Nikmatul Luailiya[2], Putri Aria Avrilian[2], Avliya Quratul Marjan[1], Dina Sugiyanti[2], Nur Hayati[2]**

[1]Nutrition Study Program, Faculty of Health Sciences, Universitas Pembangunan Nasional "Veteran" Jakarta, Depok, Indonesia
[2]Nutrition Study Program, Faculty of Psychology and Health, Walisongo State Islamic University, Semarang, Indonesia

***ARTICLE INFO***

***Received:*** *14-09-2024*
***Accepted:*** *31-12-2024*
***Published online:*** *31-12-2024*

***Correspondent:***
*Ibnu Malkan Bahrul Ilmi*
*ibnuilmi@upnvj.ac.id*

***DOI:***
*10.20473/amnt.v8i3SP.2024.218-227*

***Available online at:***
*https://e-journal.unair.ac.id/AMNT*

***Keywords:***
*Diabetes, Kefir, Cholesterol, LDL, Triglycerides*

**ABSTRACT**

**Background:** Metabolic syndrome is characterized as a collection of metabolic changes in the body that occur concurrently, including central obesity, dyslipidemia, insulin resistance, and blood pressure instability that can be improved through functional food products. One of the functional food products who prevents metabolic syndrome is honey kefir.
**Objectives:** Analyzing the effect of goat milk kefir with the addition of randu honey on lipid profiles, specifically levels of total cholesterol, triglyceride and Low Density Lipoprotein (LDL) levels in diabetic rats.
**Methods:** This study uses design true experimental research with pre-post test with control group design on 42 male Sprague Dawley rats which were randomly divided into 6 groups, namely healthy control (KS), negative control (KN), quercetin positive group (K1), metformin group (K2), kefir group (P1), and preventive group (P2). Groups KN, K1, K2, P1, and P2 were induced with metabolic syndrome. Groups KN, K1, K2, P1, and P2 were induced with streptozotocin (STZ) of 40 mg/kg BW. Group P2 was given 1.8 ml/200 g BW honey kefir simultaneously during STZ induction and High Fat Diet (HFD). The treatment of each group was carried out for 21 days. Group P1 was given 1.8 ml/200 g BW honey kefir. Measurement of lipid levels used the Cholesterol Oxidase Para Amino Phenazone (CHOD-PAP) method for total cholesterol and LDL levels, and Gliseril Phospo Para Amino Phenazon (GPO-PAP) for triglyceride levels. The research data were analyzed using One Way ANOVA, and related data were further examined using Duncan's post hoc test.
**Results:** After 21 days of intervention, P1 rats had cholesterol levels of 117.7±7.49 mg/dl; triglycerides 106.82±7.79 mg/dl; LDL 43.57±1.89 mg/dl. Total cholesterol, triglycerides and LDL levels of P1 mice were not significantly different from K1 and K2 (p-value>0.05).
**Conclusions:** Honey kefir can improve the lipid profile of diabetic mice such as quercetin and metformin.

## INTRODUCTION

Metabolic syndrome is defined as a group of symptoms related to changes in the body's metabolism which can happen simultaneously. Metabolic syndrome includes conditions such as central obesity, dyslipidemia, insulin resistance, and blood pressure instability[1]. An increase in central obesity, hypertension, dyslipidemia, and diabetes mellitus throughout the world has made metabolic syndrome a global epidemic. According to epidemiology data from the International Diabetes Federation (IDF), the prevalence of metabolic syndrome in relation to the worldwide adult population ranges from 20-25 %[2]. In Indonesia alone, the prevalence of metabolic syndrome has reached 23.34 % of the total population, with 26.6% of adult men and 21.4 % of adult women being affected[3]. As a worldwide clinical challenge, metabolic syndrome is often associated with urbanization (as a lifestyle and change in lifestyle), which has caused a rise in energy intake, obesity, and sedentary lifestyle choices[4]. Efforts to treat metabolic syndrome could be done through pharmacological or non-pharmacological methods. Metabolic syndrome could be treated non-pharmacologically through food for special dietary uses, such as probiotic drinks[5].

Food for special dietary uses are foods that can contribute towards (aside from its basic nutrition) health



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

benefits such as aiding in improving health outcomes of several diseases[6]. Probiotics are considered as food for special dietary uses[7]. In Greek, probiotics were known as a substance or organism that had health benefits for human[8]. Several studies have shown the positive effects of probiotics on treating metabolic syndrome. Probiotic intake in patients with metabolic syndrome has resulted in improvements of the patients' body mass index (BMI), blood pressure, glucose metabolism, and lipid profile[9]. Probiotics have a role in inhibiting synthesis of the HMG-CoA reductase enzyme. A decrease of this enzyme leads to a decrease in the synthesis and secretion of cholesterol[10]. Additionally, probiotics contain the metabolite product of fermented milk in the form of Conjugated Linoleic Acid (CLA), which is useful in lowering levels of triglyceride. Through CLA, triglyceride levels are reduced by increasing lipolysis and beta oxidation in fatty acids[11].

Kefir is one of many products that contain probiotics[12]. Kefir is made from the inoculation of kefir starters, which are Lactic Acid Bacteria (LAB) and yeast that undergoes the process of fermentation[13]. A number of studies have shown the positive effects of kefir as an antioxidant, immunomodulator, anti-dyslipidemia, and anti-inflammatory, which reduces levels of cholesterol and improves lactose intolerance[14]. The addition of randu flower honey in kefir made from goat's milk has given an increase in aspects such as nutritional value, taste and antioxidant activity[15]. Honey is rich in flavonoid content, which works to reduce the total cholesterol and triglyceride content[16]. Kefir, added with randu flower honey (honey kefir), contains up to 26 mg QE/100 g of flavonoids.

The effects of kefir in improving lipid profiles has been thoroughly researched. Studies using kefir made with goat's milk, with the addition of porang/konjac flour, has shown a decrease in total triglyceride and cholesterol levels, and immunomodulating effects that lower the number of neutrophils and increases lymphocytes in rats suffering from metabolic syndrome[17]. Moreover, the intervention of kefir added with pisang batu (a type of seeded banana) throughout 3 weeks has shown a decrease in LDL cholesterol, total cholesterol levels and triglyceride, as well as an increase in levels of HDL cholesterol in rats with metabolic syndrome[10]. For this reason, a study on kefir made with goat's milk with the addition of randu flower honey (honey kefir) is needed, to research on its improvement of the lipid profile due to its probiotic and flavonoid content. Specifically, this study was conducted in order to analyze the changes in total cholesterol, triglyceride and LDL levels in Sprague Dawley rats when given randu honey kefir.

## METHODS

The researcher has used an experimental method with a randomized controlled pre-post test design model in this study, with the population consisting of male Sprague Dawley rats, aged 8-10 weeks, with a body weight of 120-150 g. The rats were obtained from the iRATco Laboratorium in Bogor. The sample was determined based on World Health Organization (WHO) standards, which stated that at least 5 test animals were needed in each group[18]. In addition to a reserve of two animals, anticipating the possibility of test animals dying during research (dropping out)[19]. Therefore, the total number of test animals in each group is 7, with a total of 42 test animals used in this study. This study had received ethical approval from the UPNVJ Ethical Committee (KEP UPNVJ), with serial number 350/VIII/2024/KEP on August 1st, 2024.

### Honey Kefir Production

The production of honey kefir, as well as blood glucose testing was done at the iRATco Laboratorium in Bogor. Honey kefir is made up of three main ingredients, which are goat's milk, kefir seeds, and randu flower honey. Honey kefir is made using a simple procedure, based on an existing procedure with several modified stages[20–22]. Before it is combined with other ingredients, the goat's milk is pasteurized using the double boiler method. After pasteurization, the temperature of the goat's milk would be conditioned until it reaches room temperature. Honey kefir relies on kefir grains with Lactic Acid Bacteria (LAB) content, which is added once the goat's milk has been pasteurized and cools down to room temperature. Randu flower honey is added to the goat's milk in a 1:3 ratio. The purpose of adding randu flower honey is to reduce any goaty/gamey odor, and adds organoleptic value, while also increasing the content of antioxidants in the honey kefir product. After honey is added to the kefir, it is then fermented in a closed, dark and non-humid container for 24 hours. The following steps were carried out:



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

The following is documentation for the equipment used:


(a) Glass Beaker


(b) Heater


(c) Thermometer


(d) Container (Used for Fermentation)


(e) Tube


(f) Sieve


(g) Scale


(h) Wooden Spatula (for Stirring)

**Figure 1.** Equipment Used in Making Honey Kefir

The following is documentation for the ingredients used:


(a) Goat's Milk


(b) Randu Flower Honey


(c) Kefir Grains

**Figure 2.** Ingredients Used in Making Honey Kefir

The following is documentation for the production of honey kefir:


(a) Preparation of Equipment and Ingredients


(b) Pasteurization for 15 Seconds at 72℃ using the Double Broiler Method


(c) Cooling the Goat's Milk at Room Temperature (±27℃ )



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

(d) Adding Kefir Grains with a Ratio of: 50 g of Kefir Grains per 1000 ml of Goat's Milk

(e) Adding Randu Flower Honey with a Honey to Goat's Milk Ratio of 1:3

(f) Combining All the Ingredients

(g) Fermentation of Honey Kefir for 24 Hours in a Closed, Dark, and Non-Humid Container

**Figure 3.** Documentation of the Honey Kefir Production Process

**Preparation of Test Animals**

All test animals were acclimated for 7 days in order for them to adjust to the new environment, as well as to minimize stress before any testing was conducted[23]. The animals were cared for using a system of individually ventilated cages at 22 degrees celsius (with an added range of 3 degrees celsius), and a humidity ranging from 50-60 %. Regarding exposure to light, the researchers had provided 12 hours of darkness and 12 hours of light for the rats, with food and water given ad libitum. The rats were given the standard feed with a crude protein content of 18 %[24].

A total of 42 test animals were used, which were put into 6 groups, namely KS, KN, K1, K2, P1 and P2. KS had consisted of healthy rats who were only given the standard feed throughout the research. KN were sickly rats that only received induction of metabolic syndrome, K1 were rats with metabolic syndrome that were given a dose 15 mg/kg of body weight dose of quercetin, K2 were rats with metabolic syndrome that were given a 62.5 mg/kg of body weight dose of metformin, P1 were rats with metabolic syndrome that were given a daily 1.8 ml/200g of body weight dose of honey kefir, while P2 were rats that were given a daily dose of 1.8 ml/200g of body weight dose of honey kefir since the start of the research, alongside the induction of metabolic syndrome.

The doses of honey kefir were calculated based on previous studies that had shown 70 kg of human body weight had amounted to 200 g of body weight in test animals (rats) by multiplying the conversion factor. This equation produced a conversion factor of 0.018, in accordance with the human to animal conversion table[23]. In humans with a body weight of 70 kg, the dose of kefir needed was 100 ml[25]. which then converted to the daily dose of 1.8 ml/200 g of the rat's body weight given to test groups P1 and P2. Honey kefir was given once a day in the afternoon through a gastric probe. Doses of honey kefir were administered for 21 days. The test animals' body weight is then measured three times a day to see if any adjustments were needed to the doses. Throughout the study, test animals were given a standard 18% rodent feed and water ad libitum.

**Clinical Testing**

This study had conducted clinical testing for 3 elements, which were total cholesterol, LDL and triglyceride levels. The first phase of testing was done through a blood sample, which was conducted after the rats were given HFD and a pre-test induction of STZ, as well as a post-test intervention through the plexus retroorbitalis, to analyze levels of triglyceride and total cholesterol. Triglyceride levels were determined through the GPO-PAP method with a wavelength of 505 nm, while cholesterol and LDL were determined through the CHOD-PAP method with a wavelength of 500 nm. Before testing for said elements, blood plasma was centrifuged for 15 minutes at 3000 rpm.



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

**Data Analysis**

Data analysis of lipid profiles, namely triglyceride levels, LDL and total cholesterol were processed using SPSS. The researcher had used the Shapiro-Wilk test in order to determine the normality of data, due to it consisting of less than 50 items. Afterwards, a Lavene's test was done to determine the homogeneity of data. Any differences in levels of triglyceride, LDL cholesterol or total cholesterol was analyzed through one-way ANOVA testing, or the alternative Kruskall-Wallis method if the conditions for one-way ANOVA testing was not fulfilled. Significant differences in groups were determined using a Duncan test, after one-way ANOVA testing or a post-hoc Mann-Whitney test after using the Kruskal-Wallis method. Any significant differences were determined through (p-value<0.05).

## RESULTS AND DISCUSSIONS

**Test Animals (Rats) Body Weight**

The researchers had measured the rats' body weight once every 3 days in order to determine the dose of STZ and kefir. Homogeneity testing of body weight before and after the induction of metabolic syndrome had shown a p-value<0.05, which meant a massive variance in data, therefore a Kruskal-Wallis test was conducted. Results of Kruskal-Wallis testing found that the difference in body weight between groups had stayed the same (p-value>0.05). After administering honey kefir, the change in body weight was analyzed using Kruskal-Wallis testing, which found that the differences in the rats' body weight between groups KN, KS, K1, K2, P1 and P2 was (p-value<0.001). The highest increase in body weight on average was recorded in groups KS and KN, while the lowest increase of body weight on average was in group P2.

**Conditioning Rats with Metabolic Syndrome**

Conditioning of rats to develop metabolic syndrome was done through the administration of HFD and STZ injections. HFD and STZ clinically manifests in the form of fat buildup, insulin resistance and insulin deficiency. After 60 days of conditioning, the rats obtained had shown signs of metabolic syndrome, with the following biochemical data:

**Table 1.** Average results of triglyceride, LDL cholesterol and total cholesterol after pre-test conditioning of metabolic syndrome.

| Group | Triglyceride (mg/dl) | Cholesterol (mg/dl) | LDL (mg/dl) |
|---|---|---|---|
| KN | 133.40±6.66$^{b}$ | 147.42±5.12$^{b}$ | 51.49±3.83$^{b}$ |
| KS | 98.63±6.95$^{a}$ | 107.37±5.27$^{a}$ | 38.56±2.78$^{a}$ |
| K1 | 134.23±5.41$^{b}$ | 147.56±2.57$^{b}$ | 50.72±2.04$^{b}$ |
| K2 | 148.13±12.49$^{c}$ | 148.03±4.45$^{b}$ | 50.19±1.41$^{b}$ |
| P1 | 139.17±7.01$^{b,c}$ | 146.74±2.96$^{b}$ | 55.41±3.20$^{c}$ |
| P2 | 101.85±12.36$^{a}$ | 146.39±2.62$^{b}$ | 38.72±2.03$^{a}$ |

Normal levels of triglyceride in rats ranges from 20-114 mg/dl[25]. Groups KN, K1, K2 and P1 had shown an average triglyceride level of >114 mg/dl, while groups KS and K2 had average triglyceride levels of <114 mg/dl. Normal levels of fasting LDL in rats ranges from 7-27.2 mg/dl[25]. Based on the data above, it can be concluded that all test rats had an above average level of LDL. Although, rats from groups KS and P2 consistently had lower levels. The test rats were then given a high-fat feed following the injection of streptozotocin, which caused the rats to develop hypertriglyceridemia and an increase in LDL, which fulfilled the conditions of metabolic syndrome.

The provision of high-fat food for 60 days, as well as the 40 mg/kg of body weight streptozotocin injections were modeled after a diet that best described DM pathogenesis in humans. Several groups had developed metabolic syndrome due to the high-fat feed and STZ injections, namely the groups KN, K1, K2 and P1. The provided high-fat feed had caused the rats to develop a resistance to insulin, while the STZ injection had cytotoxic properties towards the pancreas' β cells, which would cause stable hyperglycemia, akin to the effects of type 2 diabetes mellitus. This model would clinically manifest in fat buildup, insulin resistance and insulin deficiency[26]. Insulin resistance is related to an incident of diabetes mellitus as one of the symptoms of metabolic syndrome.

Insulin resistance in fat tissue leads to insulin being unable to prevent lipolysis. Lipolysis then causes a rise in free fatty acids (FFA) as well as the inhibition of the antilipolytic effects of insulin. FFA then increases the activation of kinate proteins within the liver, and stimulates reactions of gluconeogenesis and lipogenesis. Lipogenesis results in the re-esterification of FFA in the form of VLDL[27]. In large quantities, VLDL is transported by triglyceride to be converted into HDL. Through Cholesterol Ester Transfer Proteins (CEPT), HDL is then exchanged and transported by esterase cholesterol and converted into LDL. This causes a decrease in HDL levels[28]. Other than its effects on the liver, FFA also increases the activation of kinase proteins in muscle, which results in lower levels of glucose absorption. FFA also has lipotoxic properties towards the pancreas' beta cells, which results in lower levels of insulin production[29].

Levels of triglyceride, total cholesterol and LDL after the intervention (post-test) were measured on the 93$^{rd}$ day of research. Measurements were done in order to determine the effectiveness of honey kefir towards the total level of cholesterol, triglyceride and LDL after the rats had developed metabolic syndrome. Intervention was done in 6 groups for 21 days. Analyzing the levels of triglyceride, LDL and total cholesterol yields the results seen below:



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

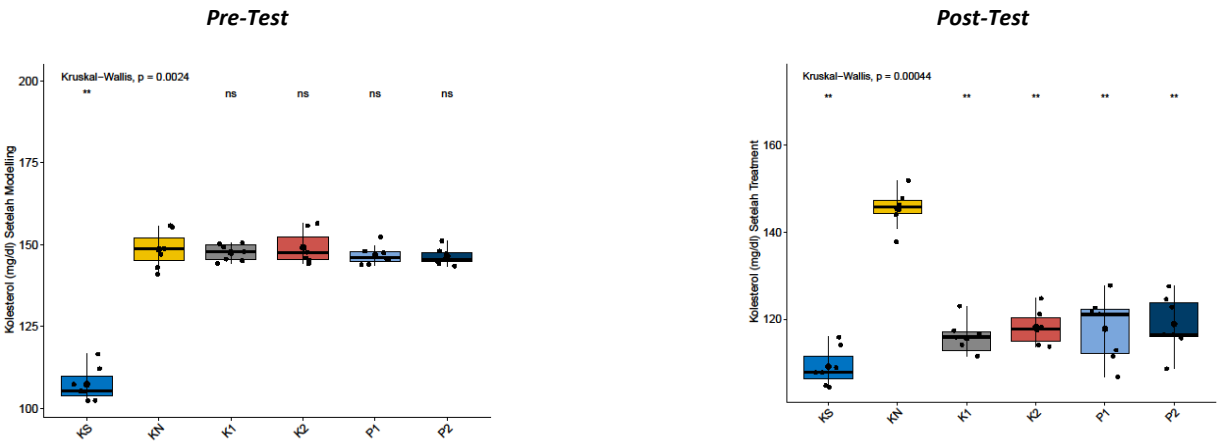


**Figure 4.** Total cholesterol levels before (pre-test) and after (post-test) intervention

Based on the figure above, there were no real differences (p-value>0.05) found in the average level of cholesterol, in every test group apart from KS. After receiving intervention for 21 days, cholesterol levels seemed to significantly decrease (p-value<0.05) in groups K1, K2, P1 and P2, nearing levels found in KS. Groups given randu honey kefir showed results that aligned with positive test groups K1 and K2, with the average value of total cholesterol being 117.77±7.49 mg/dl in P1, and 118.87±6.49 in P2. No differences in cholesterol levels were found between groups P1, K1 and K2 according to the Duncan test.

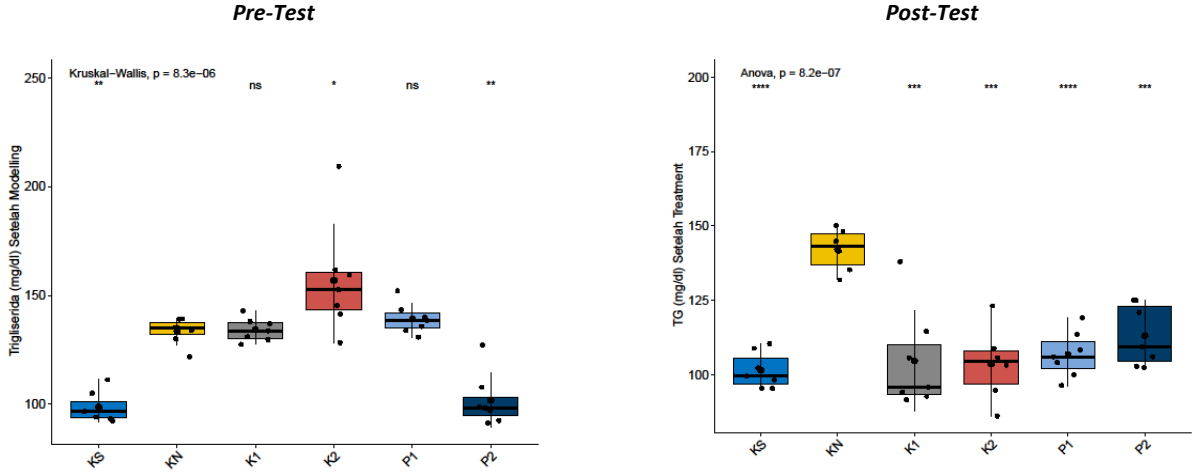


**Figure 5.** Triglyceride levels before (pre-test) and after (post-test) intervention

According to pre-test data, the highest levels of triglyceride were found in groups KN, K1, K2 and P1, and had remained significantly the same (p-value>0.05). Levels of triglyceride found in group K2 showed no significant differences from group KS (p-value<0.05). Honey kefir administered in group P2 was able to act and prevented the rise of triglyceride levels. After receiving intervention for 21 days, triglyceride levels significantly decreased in groups K1 (104.68±16.89 mg/dl), K2 (103.64±12.58 mg/dl) and P1 (106.82±7.79 mg/dl), with said results being realistically equal (p-value>0.05) to KS (101.56±6.07 mg/dl) and P2 (113.12±10.28 mg/dl).



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

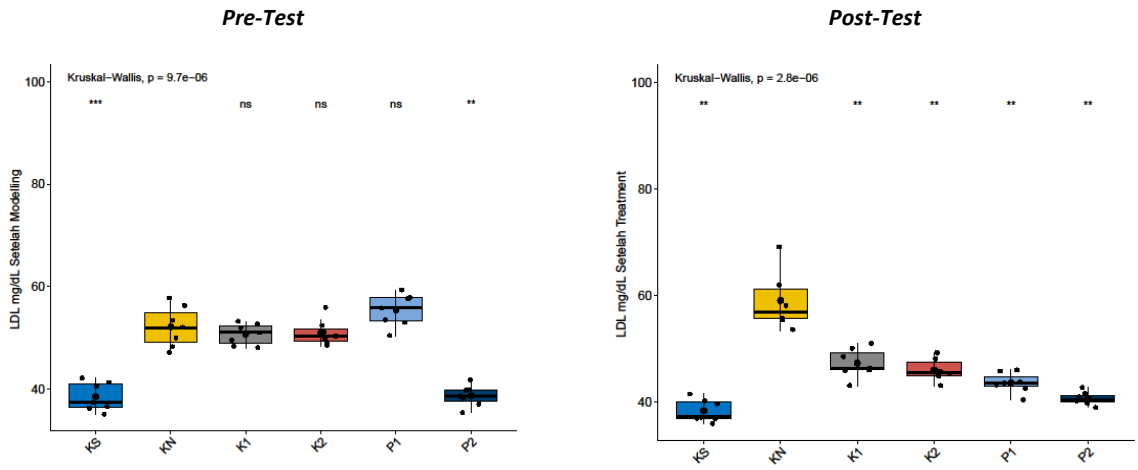


**Figure 6.** LDL levels before (pre-test) and after (post-test) intervention

Levels of LDL prior to intervention showed results that aligned with levels of triglyceride, with the lowest levels of LDL found in groups KS and P2. Groups KN, K1, K2 and P1 had LDL levels that were realistically no different from one another (p-value<0.05). Honey kefir administered in group P2 had managed to prevent the rise of LDL levels. After receiving intervention for 21 days, a significant decrease of LDL levels were found (p-value<0.05) in groups K1, K2 and P1. P1 (43.575±1.89 mg/dl), a group that was administered with honey kefir, had shown better results than positive control groups K1 (47.32±2.76 mg/dl) and K2 (45.97±2.28 mg/dl), although based on statistic tests said results showed no real difference (p-value>0.05).

Kefir is a product of fermentation that could be an alternative in preventing and treating metabolic syndrome, as well as other accompanying disorders[30]. In a study done by Kardina *et al.,* (2023) intervention using milk kefir added with buckwheat (sorgum) flour showed a decrease in total cholesterol levels between three test groups. After 21 days of intervention, group KS had experienced an increase of total cholesterol alongside levels of LDL. This condition is associated with enzymatic, pancreatic mRNA lipase, and lipid absorption which decreases as the rats age, which causes a rise in total cholesterol and LDL levels[32]. Due to aging, the group KS had experienced an increase in triglyceride, which is associated with lipid degradation caused by beta oxidation[33]. Meanwhile, the group KN had experienced an increase in total cholesterol, LDL and triglyceride levels as a consequence of the higher fat feed within 60 days following the STZ induction[34].

Changes in lipid fraction, in the form of decreasing levels of total cholesterol, LDL cholesterol and triglyceride had occurred in positive test groups (K1 and K2). Quercetin which was administered to the K1 group had decreased levels of total cholesterol and LDL through reducing the secretion of apolipoprotein in CaCo-22 cells, slowing down the activity of Microsomal Triglyceride Transfer Proteins (MTP) which inhibited the formation of lipoprotein, transferred lipids to APO B molecules, inhibition of cholesterol through HMG-KoA reductase[35,36], and modulation of microbiota in the intestine in order to reduce levels of triglyceride[37]. Metformin in the K2 group had reduced cholesterol by being an activator of the Adenosine-Monophosphate-Activated-Protein (APMK), which results in the inhibition of the acetyl coenzyme carboxylase. Acetyl coenzyme carboxylase plays a role in fat metabolism. Increased oxidation of fatty acids, in addition with the inhibition of enzymes that play a role in lipogenesis, had resulted from this mechanism[38]. Levels of triglyceride had also decreased due to metformin by inhibiting the synthesis of triglycerides[39].

The reduction of total cholesterol, LDL and triglyceride in groups P1 and P2 were initiated with the provision of honey kefir, a food for dietary use. The decrease in cholesterol levels was associated with a decrease in both triglyceride and LDL cholesterol levels[40] If abundant levels of triglyceride were found, a number of which would be diverted to the liver, which receives the components needed to produce cholesterol[41]. As a transporter of cholesterol, LDL levels would also increase. Likewise, if triglyceride levels decrease, total cholesterol and LDL levels would follow suit. In a study done by Maryusman *et al.,* (2020), it was concluded that a decrease in triglyceride and blood cholesterol, within groups of test rats experiencing metabolic syndrome, was due to the administering of synbiotic kefir made from pisang batu flour. Furthermore, the results of this study has been complemented by another study which involved synbiotic goat's milk kefir added with porang/konjac glucomannan, which resulted in a reduction of total cholesterol and triglyceride levels in rats that were given HFFD feed[17].

Honey kefir is a probiotic product with a BAL content reaching up to 3.50x1010 CFU/ml. The administering of honey kefir had resulted in a decrease of total cholesterol levels, which is attributed to its probiotic contents. Probiotics found in kefir consist of BAL, which contains Bile Salt Hydrolase (BSH) enzymes. BSH plays a role in deconjugating salt found in bile. The amount of bile acids in the liver decreases as it is excreted through feces after it has been conjugated. The amount of bile acids excreted through feces increases the synthesis of bile salts, which causes cholesterol levels to fall[43]. In addition, BAL is also able to absorb cholesterol. Cholesterol absorption in the digestive system causes a



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

decrease in blood cholesterol levels [44]. Short Chain Fatty Acids (SCFAs) such as acetic, butyric and propionic acids are also produced by probiotics, which are responsible for the inhibition of the enzyme HMG-CoA[45].

Kefir contains amino acids that play a role in reducing the expression of SREBP-1c, in order to suppress Fatty Acid Synthase (FAS) and control lipogenesis[46]. Aside from amino acids, kefir also suppresses PPARγ, which is responsible for regulating the adipogenesis process (formation of adipocytes), which results in lipid oxidation and inhibits the process of adipogenesis and lipogenesis within adipocytes. Suppression of lipogenesis rates causes a decrease in fat buildup and size of adipose tissue. In addition, CLA found in kefir increases lipolysis and beta-oxidation of fatty acids, which reduces the level of triglycerides[47].

Randu flower honey, which was added to the kefir, contains ascorbic acids, flavonoids, phenolic acids, carotenoids, alongside amino acids and protein. Honey kefir contains up to 26 mg QE/100 g of flavonoids. Apart from probiotics, flavonoids are also responsible for inhibiting activity of the HMG KoA reductase enzyme[48,49]. As an exogen antioxidant, flavonoids donate H+ ions, which reduces oxidative stress. Repairing oxidative stress increases the sensitivity of insulin in glucose uptake. Improvements of insulin sensitivity results in repairs of the lipid metabolism.

The advantage of this study is that it uses a preventative and curative design. Both of these designs are used in order to determine the role of kefir as a method of prevention, or to treat metabolic syndrome (especially on the issue of lipid levels). The weakness of this study is that it did not test for the optimal dose of honey kefir.

## CONCLUSIONS

The administering of goat's milk kefir, added with randu flower honey (group P1) for 21 days has shown a significant decrease in total cholesterol, LDL and triglyceride levels, akin to quercetin (group K1) and metformin (group K2). The administering of goat's milk kefir added with randu flower honey alongside HFD for 60 days and STZ induction (group P2) has managed to prevent metabolic syndrome, and is more optimal in reducing levels of triglyceride and LDL compared to other groups. This has shown that honey kefir is able to act simultaneously as a preventative and curative agent for metabolic syndrome.

## ACKNOWLEDGEMENT

The author would like to thank the DRTPM KEMENDIKBUD, who have provided the basic research funding grant through the standard fundamental research scheme.

## CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The author has reported no conflicts of interest. The author alone is responsible for the contents and writing of this article. This research is funded by the Directorate of Research, Technology and Community Service (DRTPM) of the Directorate General of Higher Education from the Ministry of Education and Culture of the Republic of Indonesia, on the basis of a standard fundamental research scheme, with contract number: 002/UN.61.4/PD-PFR/2024.

## AUTHOR CONTRIBUTIONS

IMBI: conceptualization, methodology, validation, supervision, writing-review, and editing; AH: conceptualization, methodology, validation, supervision, writing-review, and editing; NL: conceptualization, investigation, methodology, formal analysis, writing-original draft, writing-editing; PAA: conceptualization, investigation, methodology, formal analysis, writing-original draft, writing-editing; AQM: conceptualization, validation; DS: conceptualization, methodology, validation; NH: conceptualization, methodology, validation.

## REFERENCES

1. Mazidi, M., Rezaie, P., Kengne, A. P., Mobarhan, M. G. & Ferns, G. A. Gut Microbiome and Metabolic Syndrome. *Diabetes Metab. Syndr. Clin. Res. Rev.* **10**, S150–S157 (2016). https://doi.org/10.1016/j.dsx.2016.01.024
2. International Diabetes Federation. IDF consensus worldwide definition of the metabolic syndrome. in (2023).
3. Orno, T. G. & Fauzi, A. Z. Deteksi Dini Sindrom Metabolik dengan Metode NCEP ATP-III pada Masyarakat Desa Waai Kabupaten Maluku Tengah. *Molucca Medica* **15**, 1–8 (2022). https://doi.org/10.30598/molmed.2022.v15.i1.1
4. Rustika, R., Driyah, S., Oemiati, R. & Hartati, N. S. Prediktor Sindrom Metabolik : Studi Kohor Prospektif Selama Enam Tahun di Bogor, Indonesia. *Media Penelit. Dan Pengemb. Kesehatan.* **29**, 215–224 (2019). https://doi.org/10.22435/mpk.v29i3.654
5. Xavier-Santos, D., Bedani, R., Lima, E. D. & Saad, S. M. I. Impact of Probiotics and Prebiotics Targeting Metabolic Syndrome. *J. Funct. Foods* **64**, 103666 (2020). https://doi.org/10.1016/j.jff.2019.103666
6. Topolska, K., Florkiewicz, A. & Filipiak-Florkiewicz, A. Functional Food—Consumer Motivations and Expectations. *Int. J. Environ. Res. Public. Health* **18**, 5327 (2021). https://doi.org/10.3390/ijerph18105327
7. Damián, M. R. *et al.* Functional Foods, Nutraceuticals and Probiotics: A Focus on Human Health. *Microorganisms* **10**, 1065 (2022). https://doi.org/10.3390/microorganisms10051065
8. Zendeboodi, F., Khorshidian, N., Mortazavian, A. M. & da Cruz, A. G. Probiotic: Conceptualization from A New Approach. *Curr. Opin. Food Sci.* **32**, 103–123 (2020). https://doi.org/10.1016/j.cofs.2020.03.009
9. Tenorio-Jiménez, C., Martínez-Ramírez, M. J., Gil, Á. & Gómez-Llorente, C. Effects of Probiotics on Metabolic Syndrome: A Systematic Review of Randomized Clinical Trials. *Nutrients* **12**, 124 (2020). ttps://doi.org/10.3390/nu12010124
10. Khoiriyah, D., Maryusman, T. & Herlina, S. Pengaruh Sinbiotik Kefir Pisang Batu terhadap



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

Kadar Kolesterol-LDL dan Kolesterol-HDL Tikus Model Sindrom Metabolik. *J. Bioteknol. Dan Biosains Indones.* **7**, 280–288 (2020). https://doi.org/10.29122/jbbi.v7i2.3781

11. Sari, F. N. P. & Pramono, A. Pengaruh Pemberian Kefir Susu Sapi terhadap Kadar Trigliserida Tikus Jantan Sprague Dawley. *J. Nutr. Coll.* **1**, 322–326 (2012).
12. Elbashiti, T. A. & Zabut, A. M. (PDF) Effect of Kefir Intake on Growth Performance and Some Biochemical Profiles Among Domestic Rabbits. *ResearchGate* (2024) doi:10.20959/wjpps20173-8801. DOI: 10.20959/wjpps20173-8801
13. Martharini, D. & Indratiningsih, I. Kualitas Mikrobiologis dan Kimiawi Kefir Susu Kambing dengan Penambahan Lactobacillus acidophilus FNCC 0051 dan Tepung Kulit Pisang Kepok (Musa Paradisiaca). *Agritech* **37**, 23 (2017). https://doi.org/10.22146/agritech.17002
14. Bengoa, A. A., Iraporda, C., Garrote, G. L. & Abraham, A. G. Kefir Micro-Organisms: Their Role in Grain Assembly and Health Properties of Fermented Milk. *J. Appl. Microbiol.* **126**, 686–700 (2019). https://doi.org/10.1111/jam.14107
15. Hardiansyah, A. & Kusuma, H. H. Optimalisasi Kualitas Organoleptik dan Aktivitas Antioksidan Kefir Susu Kambing dengan Penambahan Madu Lokal Bunga Randu. *J. Nutr. Coll.* **11**, 278–284 (2022). https://doi.org/10.14710/jnc.v11i4.34506
16. Kusuma, A. M., Asarina, Y., Rahmawati, Y. I. & Susanti, S. Efek Ekstrak Bawang Dayak (Eleutherine palmifolia (L.)Merr) dan Ubi Ungu (Ipomoea batatas L) terhadap Penurunan Kadar Kolesterol dan Trigliserida Darah pada Tikus Jantan. *J. Kefarmasian Indones.* 108–116 (2016) doi:10.22435/jki.v6i2.2925. https://doi.org/10.22435/jki.v6i2.2925
17. Nurliyani, N., Harmayani, E. & Sunarti, S. Goat Milk Kefir Supplemented with Porang Glucomanna Improves Lipid Profile and Haematological Parameter in Rat Fed High Fat and High Fructose Diet. *Romanian J. Diabetes Nutr. Metab. Dis.* **25**, 11–21 (2018). doi: 10.2478/rjdnmd-2018-0002
18. WHO. *General Guideline for Methodologies on Research and Evaluation of Traditional Medicine*. (WHO, Geneva, 2001).
19. Zaki, I., Johan, A. & W, N. S. Pengaruh Pemberian Jus Mangga terhadap Profil Lipid dan Malondialdehyde pada Tikus yang Diberi Minyak Jelantah. *J. Gizi Indones. Indones. J. Nutr.* **3**, 108–115 (2015). https://doi.org/10.14710/jgi.3.2.108-115
20. Lindawati, S. A., Sriyani, N. L. P., Hartawan, M. & Suranjaya, I. G. Study Mikrobiologis Kefir dengan Waktu Simpan Berbeda. *Maj. Ilm. Peternak.* **18**, (2015).
21. Muntafiah, A., Sunarti & Nurliyani. Potensi Antihiperglikemia Kefir Berbasis Susu Kambing dan Kedelai pada Tikus Model DM Tipe 2. *Mandala Health* **8**, 612–621 (2015).
22. Nurfadilah, S. Optimasi Suhu Inkubasi terhadap Mutu Kefir Ekstrak Bunga Rosella (Hibiscus sabdariffa L). *Farmakol. J. Farm.* **19**, 191–203 (2022). https://doi.org/10.56730/farmakologika.v19i2.413
23. Maryusman, T., Dien, C. D. & Mail, S. H. Uji Efektifitas Sinbiotik Kefir Tepung Pisang Batu terhadap Kadar Glukosa Darah dan Kadar Profil Lipid Tikus Model Sindrom Metabolik. *Media Gizi Indones.* **16**, 296–305 (2021). https://doi.org/10.20473/mgi.v16i3.296–305
24. Nurmasitoh, T. & Pramaningtyas, M. D. Honey Improves Lipid Profile of Diet-Induced Hypercholesterolemic Rats. *Universa Med.* **34**, 177–186 (2015). https://doi.org/10.18051/UnivMed.2015.v34.177-186
25. Rahmawati, F. C., Djamiatun, K. & Suci, N. Pengaruh Yogurt Sinbiotik Pisang terhadap Kadar Glukosa dan Insulin Tikus Sindrom Metabolik. *J. Gizi Klin. Indones.* **14**, 10–18 (2017). https://doi.org/10.22146/ijcn.19379
26. Chen, D. & Wang, M.-W. Development and Application of Rodent Models for Type 2 Diabetes. *Diabetes Obes. Metab.* **7**, 307–317 (2005). https://doi.org/10.1111/j.1463-1326.2004.00392.x
27. Bermúdez-Cardona, J. & Velásquez-Rodríguez, C. Profile of Free Fatty Acids and Fractions of Phospholipids, Cholesterol Esters and Triglycerides in Serum of Obese Youth with and without Metabolic Syndrome. *Nutrients* **8**, 54 (2016). https://doi.org/10.3390/nu8020054
28. Rajab, A. A., Nuhriawangsa, A. M. P. & Rahardjo, S. S. Efek Kombinasi Bubuk Mengkudu dan Kelor terhadap Glukosa Darah Puasa Tikus DMT2 Dislipidemia. *GIZI Indones.* **46**, 57–66 (2023). https://doi.org/10.36457/gizindo.v46i1.765
29. Anggraini, D. Tinjauan Pustaka: Sindrom Metabolik. *J. Syntax Admiration* **5**, 836–851 (2024). https://doi.org/10.46799/jsa.v5i3.1061
30. Pimenta, F. S. *et al.* Mechanisms of Action of Kefir in Chronic Cardiovascular and Metabolic Diseases. *Cell. Physiol. Biochem.* **48**, 1901–1914 (2018). https://doi.org/10.1159/000492511
31. Kardina, R. N. *et al.* Effectiveness of Milk Kefir Administration with the Addition of Sorghum Flour Against Total Cholesterol Levels, and Blood Glucose Levels in Obesity-Induced Mice. *Bali Med. J.* **12**, 3287–3289 (2023).
32. Parini, P., Angelin, B. & Rudling, M. Cholesterol and Lipoprotein Metabolism in Aging: Reversal of Hypercholesterolemia by Growth Hormone Treatment in Old Rats. *Arterioscler. Thromb. Vasc. Biol.* **19**, 832–839 (1999). https://doi.org/10.1161/01.ATV.19.4.832
33. Zhao, L. *et al.* Evidence for Association of Mitochondrial Metabolism Alteration with Lipid Accumulation in Aging Rats. *Exp. Gerontol.* **56**, 3–12 (2014). https://doi.org/10.1016/j.exger.2014.02.001



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*

34. Liu, J., Han, L., Zhu, L. & Yu, Y. Free Fatty Acids, Not Triglycerides, are Associated with Non-Alcoholic Liver Injury Progression in High Fat Diet Induced Obese Rats. *Lipids Health Dis.* **15**, 27 (2016). https://doi.org/10.1186/s12944-016-0194-7
35. Ilmi, M. B. I., Hardiansyah, A., Marjan, A. Q., Octaria, Y. C., Saputra, M. M., & Susilowati, F. (2024). Analysis of Total Lactic Acid Bacteria (LAB) and Organoleptic Quality of Goat Milk Kefir With the Addition of Kapok Honey (Ceiba pentandra L.). *Malaysian Journal of Medicine & Health Sciences*, *20*.).
36. Witosari, N. & Widyastuti, N. Pengaruh Pemberian Jus Daun Ubi Jalar (Ipomoea batatas (L.) Lam) terhadap Kadar Kolesterol Total Tikus Wistar Jantan (Rattus Norvegicus) yang Diberi Pakan Tinggi Lemak. (Diponegoro University, 2014). https://doi.org/10.14710/jnc.v3i4.6863
37. Kuipers, E. N. *et al.* Quercetin Lowers Plasma Triglycerides Accompanied by White Adipose Tissue Browning in Diet-Induced Obese Mice. *Int. J. Mol. Sci.* **19**, 1786 (2018). https://doi.org/10.3390/ijms19061786
38. Dini, M. I. & Susanti, E. Effect of Purple Sweet Potato Extract Administration on Total Cholesterol Level of Diabetic and High Fat Diet Wistar Rats. *PHARMADEMICA J. Kefarmasian Dan Gizi* **2**, 9–20 (2022). https://doi.org/10.54445/pharmademica.v2i1.20
39. Fitriani, A. & Padmasari, S. Analisis Potensi Interaksi Obat Antidiabetik Pada Pasien Diabetes Melitus Tipe 2 Rawat Inap RS PKU Muhammadiyah Gamping Yogyakarta. *Maj. Farm.* **18**, 37–42 (2022). https://doi.org/10.22146/farmaseutik.v18i1.71905
40. Pratiwi, V. N., Astuti, M. & Murdiati, A. Efek Pemberian Diet Beras Merah Dan Beras Putih Prapemasakan Terhadap Kadar Total Kolesterol, Trigliserida, Dan Berat Badan Tikus Hiperglikemia. *J. Teknol. Pangan* **12**, 17–23 (2019). https://doi.org/10.33005/jtp.v12i2.1285
41. Tandra, dr H. *Kolesterol dan Trigliserida*. (Gramedia Pustaka Utama, 2024).
42. Maryusman, T., Imtihanah, S. & Firdausa, N. I. Kombinasi Diet Tinggi Serat Dan Senam Aerobik Terhadap Profil Lipid Darah Pada Pasien Dislipidemia. *GIZI Indones.* **43**, 67–76 (2020). https://doi.org/10.36457/gizindo.v43i2.354
43. Lee, D. K. *et al.* Lactic Acid Bacteria Affect Serum Cholesterol Levels, Harmful Fecal Enzyme Activity, and Fecal Water Content. *Lipids Health Dis.* **8**, 21 (2009). https://doi.org/10.1186/1476-511X-8-21
44. Yuniastuti, A. Pengaruh Pemberian Susu Fermentasi Lactobacillus Casei Strain Shirota Terhadap Perubahan Kadar Fraksi Lipid Serum Tikus Hiperkolesterolemi (Effect of Administration of Lactobacillus Casei Strain Shirota Fermented Milk on the Alteration of Serum Lipid Fraction of Hypercholesterolemic Rats). (Program Pasca Sarjana Universitas Diponegoro, 2004).
45. Adnan, M. L. Efek Konsumsi Kefir Terhadap Dislipidemia Terkait Sindrom Metabolik: Sebuah Tinjauan Pustaka. *Khazanah J. Mhs.* **13**, (2021). doi: 10.20885/khazanah.vol13.iss1.art4
46. Radiati, L. E., Hati, D. L., Andarini, S., Handayani, D. & Rosyidi, D. Potensi Whey Kefir Susu Kambing Sebagai Anti-Obesitas Melalui Penghambatan Sintesis Lipid dan Aktivitas Phosphoenolpyruvate Carboxykinase (PEPCK) pada Sel Model Adiposit 3T3-L1. *Indones. J. Hum. Nutr.* **9**, 207–207 (2022). doi : 10.21776/ub.ijhn.2022.009.02.9
47. Williams, L. *C.L.A : The Essential Nutrients for Cutting Cancer Risk, Reducing Body Fat, and Providing Antioxidant Properties (Woodland Health Series)*. (Woodland Pub, 1997).
48. Fitriarini, S. & Rahayuningsih, H. M. Perbedaan Pengaruh Antara Ekstrak Dan Rebusan Daun Salam (Eugenia Polyantha) Dalam Pencegahan Penurunan Kadar Kolesterol Hdl Pada Tikus Sprague Dawley. *Journal of Nutrition College* vol. 3 184–191 (Diponegoro University, 2014). https://doi.org/10.14710/jnc.v3i1.4557
49. Rusmini, H., Marlina, D. & Lestari, P. Pengaruh Flavanoid dalam Ekstrak Mentimun (Cucumis sativus L) terhadap Kadar Kolesterol Total Darah Mencit (Mus musculus L) yang Mengkonsumsi Makanan Cepat Saji. *J. Ilmu Kedokt. Dan Kesehat.* **6**, 166–175 (2019). https://doi.org/10.33024/jikk.v6i3.2087



***How to cite:*** *Ilmi, I. M. B., Hardiansyah, A., Luailiya, N., Avrilian, P. A., Marjan, A. Q., Sugiyanti, D., & Hayati, N. (2024) The Effect of Honey Kefir on the Lipid Profile of Sprague Dawley Rats with Metabolic Syndrome: Pengaruh Pemberian Kefir Madu terhadap Profil Lipid Tikus Galur Sprague Dawley Sindroma Metabolik. Amerta Nutrition, 8(3SP), 218–227.*