

Minyak Ayam sebagai Minyak Goreng Baru : Pengaruhnya pada Profil Lipid dan Histologi Liver pada Tikus Wistar Jantan

Chicken Oil as The New Cooking Oil: Its Effect on Lipid Profile and Liver Histology in Male Wistar Rats

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ABSTRAK

Latar Belakang: Pemilihan minyak goreng yang tepat oleh masyarakat akan mencegah asupan lemak yang tinggi. Minyak ayam sebagai minyak goreng baru, yang mulai digunakan oleh sebagian orang sebagai pengganti minyak sawit, perlu diteliti efeknya terhadap kesehatan.

Tujuan: Penelitian ini bertujuan untuk melihat dan membandingkan perubahan profil lipid dan histologi liver pada tikus wistar jantan yang diberi minyak ayam dan minyak sawit.

Metode: Penelitian ini merupakan penelitian eksperimental dengan menggunakan desain A two-group pretest-posttest. Tikus wistar jantan (n = 10) dipisahkan menjadi dua kelompok secara acak, yaitu kelompok minyak ayam dan kelompok minyak sawit. Setiap tikus dalam kelompok diberi minyak dengan dosis 1 mL/hari selama empat minggu. Sebelum perlakuan, kadar profil lipid diukur, dan setelah empat minggu perlakuan, kadar profil lipid dan histologi liver diperiksa.

Hasil: Pemberian 1 mL/hari minyak pada setiap kelompok selama empat minggu menurunkan kadar kolesterol total, HDL, LDL pada ketiga kelompok juga meningkatkan kadar trigliserida pada ketiga kelompok, tetapi perbedaan tidak bermakna antar kelompok. Penelitian ini juga menunjukkan pembentukan steatosis pada semua kelompok, tetapi masih steatosis mikrosesuler ringan-sedang, dan perbedaan tidak bermakna antar kelompok.

Kesimpulan: Konsumsi 1 mL/hari minyak ayam selama empat minggu memiliki pengaruh yang sama terhadap profil lipid dan histologi hati dengan konsumsi 1 mL/hari minyak sawit dengan durasi yang sama. Kami mengusulkan penelitian lebih lanjut dengan memberikan intervensi minyak ayam pada tikus dalam waktu yang lebih lama.

Kata kunci: Minyak Kulit Ayam, Minyak Sawit, Profil Lipid, Steatosis, Asam Lemak

ABSTRACT

Background: The selection of the appropriate cooking oil by society will prevent too high a fat intake. Chicken oil as a new cooking oil that is starting to be used by some people as a substitute for palm oil, needs to be studied for its health effects.

Objectives: This study aimed to see and compare changes in lipid profiles and liver histology in male Wistar rats fed with chicken oil and palm oil.

Methods: The study was an experimental study with A two-group pretest-posttest design. Male Wistar rats (n = 10) were separated into two groups randomly, namely the chicken oil group and the palm oil group. Each rat in the group was given oil at a dose of 1 mL/day for four weeks. Before treatment, lipid profile levels were measured, and after four weeks of treatment, lipid profile levels and liver histology were examined.

Results: Administration of 1 mL/day of oil in each group for four weeks diminished total cholesterol, HDL, LDL levels at the two groups also increased triglyceride levels in the two groups, but non-significant differences among groups. This research also showed the formation of hepatic steatosis in all groups, but still mild-moderate microvesicular steatosis, and non-significant differences among groups.

Conclusion: The consumption of 1 mL/day of chicken oil for four weeks has a similar effect on lipid profiles and liver histology as the consumption of 1 mL/day of palm oil with the same duration. We propose further study by administering the intervention of chicken oil to rats for a longer time.

Keywords: Chicken Skin Fat, Palm Oil, Lipid Profile, Steatosis, Fatty Acid



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INTRODUCTION

High serum cholesterol levels are closely related to the prevalence of chronic diseases like atherosclerosis, coronary syndrome, cancer, stroke, also fatty liver.¹ Various studies in both animal trials and clinical trials in humans have proven this. Several research results confirmed that an increase in serum cholesterol levels is a predisposing factor for the onset of the disease.²

Cholesterol does not always harm the body, because cell membranes and steroid hormones are consisted of cholesterol. Thus, the sufficient amount of cholesterol is needed by the body. If the amount is excessive, hypercholesterolemia will occur,³ which is indicated by increasing levels of LDL, triglycerides, and total cholesterol.⁴ Too high intake of fat, mainly saturated fat, will cause high cholesterol levels. The increase in fat consumption by the public noticed from the tendency to consume fried food.

The primary source of fat for the Indonesian people that must be of concern to the public is cooking oil. People should choose the cooking oil that is not harmful to the body. Cooking oil that has been through clinical trials is proven not to increase "bad" cholesterol levels in the blood, does not cause the formation of atherosclerosis or fatty liver, or other health problems, which is the right cooking oil for consumption. Choosing and using the right cooking oil will prevent too high a fat intake. Various types of cooking oil are circulating in the market, which is widely used by the community is palm oil.⁵ The price of palm oil is still not affordable for some people so there are still people who use coconut oil that is produced by themselves by utilizing the coconut in their garden. Recently, a new type of cooking oil, known as chicken oil, has also emerged. In our area in South Sulawesi, broiler chicken sellers produced chicken oil. Chicken skin waste is collected and used to make chicken oil. Their target buyers are sellers of fried snacks. The sellers of fried snacks feel that they benefit from using chicken oil besides that the price was more affordable than palm oil, chicken oil also adds flavor to fried foods. Currently, we can find chicken oil in e-commerce. The target buyers are those on the keto diet. They used chicken oil as a source of healthy fats. However, it was high-priced because the chicken skin raw material also has to be purchased by the seller.

There are no studies on the health effects of chicken oil. So that in this research, we will examine the impact of chicken oil on lipid profiles in the blood and fat accumulation in the liver. Also, how is the effect of chicken oil when compared to palm oil is widely consumed by people today. If this study shows that

chicken oil has the same effect as palm oil, further research on chicken oil can be accomplished because it has the potential to be a safe cooking oil for consumption at a more affordable price. However, if the study found that chicken oil had a less favorable effect than palm oil, it could be an educational material for the public not to consume chicken oil.

METHODS

Experimental Animals and Ethical Clearance

Ten rats were used for the research. The inclusion criteria were male, Wistar strains, 4-5 months of age, 280-300 grams of weight, healthy and active. Meanwhile, the exclusion criteria were rats that were sick before treatment and rats that died during the research. Before treatment, the rats were acclimatized in the cage for 14 days so that their lifestyle and diet were homogenized. The rats were given standard feed (AD2) and drinking water ad libitum before and during treatment. The cage environment was made not to be humid, with adequate ventilation and sufficient lighting where it was light for 12 hours and dark for 12 hours. Rat health was monitored every day. The Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University has confirmed the proposal and research protocol (No. 458 / UN4.6.4.5.31 / PP36 / 2020).

Chicken Oil Extraction

The chicken oil was extracted via traditional methods. This method was used by the community to produce chicken oil. 1.000-gram chicken skin (*Gallus domesticus*) cleaned and cut into small pieces, placed in a frying pan then heated for about 75 minutes on the stove over medium heat, stirring occasionally, until the chicken skin was dry, and produces a colored oil golden yellow. Remove the chicken skin, cooling the chicken oil at room temperature. Separate the oil from the sediment, then transfer it to a closed container.

Experimental design

The study was an experimental study with A *two-group pretest-posttest design*. The animal study was divided into two groups randomly (1 group consisting of 5 animals). And given different treatments for four weeks, as described below.

- 1) Group 1 given the chicken oil
- 2) Group 2 given the palm oil

The oil dosage applied in this research was the equivalent of 30 g or 30,000 mg of oil intake in humans, given to each group.⁶ Determination of the dose in rat using the Reagan-Shaw method with the formula :⁷



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$$\text{HED} = \text{Animal Dose} \left(\frac{\text{mg}}{\text{kg/bw}} \right) \times \frac{\text{Animal Km}}{\text{Human Km}}$$

In humans, the intake of cooking oil per day (Human Equivalent Dosage-HED) was 30.000 mg divided by 60 kg, which was 500.⁶ The constant factor (Km) in rats was 6. and the constant factor in adult humans were 37. so the dose in rats was 3.083 mg/kg. The rats applied had an average weight of 280 g, so that its dosage used was 863 mg/rat, or the equivalent of 0.9 mL/rat, rounded to 1 mL/rat. Chicken oil and palm oil stored at room temperature. Raw oil gave through a cannula to rats.

Body Weight

During the treatment for four weeks, the weight of the experimental animals weighed every week to saw changes in body weight.

Lipid Profile Determination

Blood samples were taken the day before treatment and at the end of the 4th week of treatment to measure the serum lipid profile. Before taking blood, the rats were fasted for about 10 to 12 hours, only given drinking water. Blood was collected into a red-cap vacutainer tube (without anticoagulant) and centrifuged at 3000 rpm for fifteen minutes to obtain serum. The levels of lipid profiles include total cholesterol, HDL, LDL, and triglycerides detected by a spectrophotometer utilizing the Quimica Clinica Aplicada S.A. Reagent kit. (QCA). The tests were fully done according to the manufacturer's guidance.

Histological Analysis through HE Staining

The termination was carried out after four weeks of treatment. The rats were sedated with ether, then terminated through a cervical dislocation (neck dislocation). The peritoneum opened, the liver was removed and then put in an organ pot containing formalin and marked with a label. After that the dehydration process with graded alcohol for 30 minutes. Then, the clearing process with pure xylol was also for 30 minutes. The next, the embedding process then the cutting process with a microtome, attaching it to the slide, staining with hematoxylin and eosin for microscopic examination. The assessment was the presence or absence of macrovesicular steatosis or microvesicular steatosis and the scoring given based on the intensity of the steatosis, ranging from mild, moderate to severe (mild +, moderate ++, severe +++).

Statistical analysis

The data were processed using SPSS software. The differences between groups were calculated using the Independent Samples T-test. The Mann-Whitney U test was used to analyze the histopathological results. To show statistically significant differences, a *p*-value <0.05 was determined.

RESULTS AND DISCUSSION

Experimental Animal Weight

Weighing results showed raising in the body weight of rats in all groups during treatment. The increase in body weight in the chicken oil group was higher than in the palm oil group, but statistically, the weight gain of the two groups was an insignificantly different (*p*=0.222) (table 1).

Table 1. The changes in Body Weight (g) after four weeks of treatment

Treatment Group	Initial Weight	Final Weight	Weight Gain
The Chicken Oil Group	289±8.22	344±33.62	55±27.61
The Palm Oil Group	288±5.70	322±26.83	34±22.19
<i>p</i> value*			.222

*Significance was tested with Independent Samples T-test (*p*<0.05)

Lipid Profile Parameters

After four weeks of treatment, the levels of total cholesterol in the two groups (Figure 1) decreased. The reduction in total cholesterol levels in the palm oil group was higher than the chicken oil group, but not significantly different from the chicken oil group (*p*=0.423) (table 2). The levels of LDL and HDL also decrease in all groups (Figure 1). The reduction in LDL levels and HDL levels at the chicken oil group was higher

than the palm oil group, but not significantly different from the chicken oil group (*p*=0.308 for LDL, *p*=0.580 for HDL) (table 2). Meanwhile, the levels of triglycerides in all groups increased (Figure 1), and the increase in triglycerides levels in the chicken oil group was higher than the palm oil group, but not significantly different from the palm oil group (*p*=0.646) (table 2).



Table 2. The changes in Serum Lipid Levels (mg/dL) after four weeks of treatment

Treatment Group	Δ Total Cholesterol	Δ LDL	Δ HDL	Δ Triglyceride
The Chicken Oil Group	20.48±6.14	7.08±0.94	5.53±5.14	55.89±35.94
The Palm Oil Group	24.84±9.79	5.94±2.15	4.08±2.27	47.01±20.95
<i>p</i> value*	.423	.308	.580	.646

*Significance was tested with Independent Samples T-test (*p*<0.05)

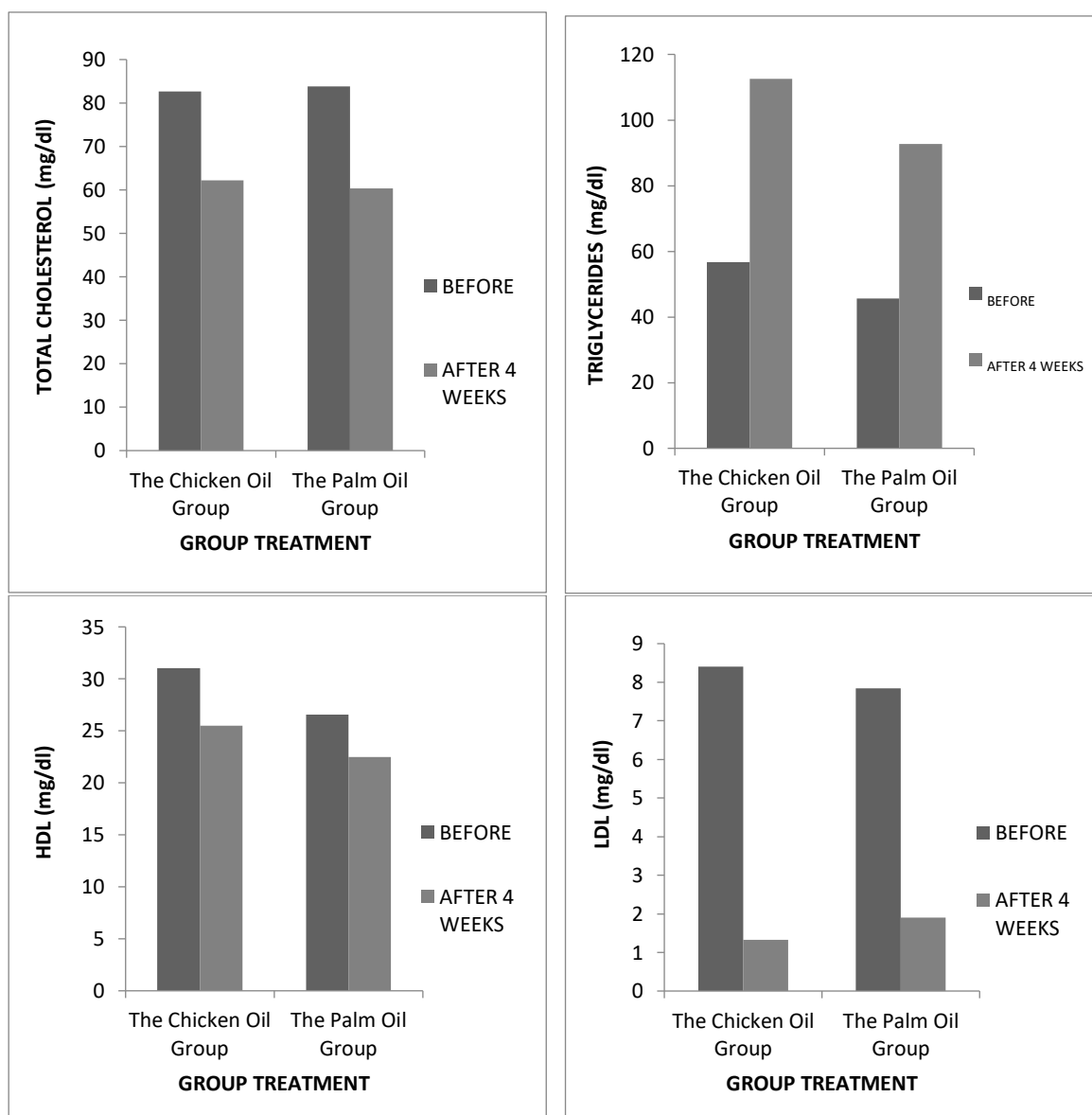


Figure 1. Serum Lipid Levels Before and After 4 Weeks of Treatment

Chicken Oil and Palm Oil groups experienced a decrease in total cholesterol, LDL, HDL levels, and an

increase in triglyceride levels. The decrease in total cholesterol levels in the palm oil group was higher than the chicken oil group, the decrease in LDL and HDL levels



in the chicken oil group was higher than the palm oil group, and the increase in triglyceride levels in the chicken oil group was higher than the chicken oil group.

The extraction of chicken oil was obtained by directly heating the chicken skin over a fire or by using a microwave. There were eleven types of fatty acids found in chicken fat oil, eight of which were saturated fatty acids (SFA), and the others were unsaturated fatty acids. Oleic acid has the highest concentration, followed by palmitic acid, palmitoleic acid, and stearic acid.⁸ No previous research has looked at the effect of chicken oil on lipid profile levels, but several studies were showing that oils containing high oleic acid have beneficial impacts on reducing the risk of cardiovascular disease because they can reduce levels of lipid profiles in the blood, mainly decreasing LDL levels.^{9,10} It was synchronous with this research, namely a decrease in total cholesterol and LDL levels in the group given the chicken oil for four weeks. Total cholesterol and LDL levels were also reduced in the group given palm oils. Palm oil was an oil with a saturated fatty acid content of 49.9% and an unsaturated fatty acid of 49.7%. Among the eight types of fatty acids found in palm oil, palmitic acid, which was a saturated fatty acid, has the highest concentration at 44.0%, then oleic acid, which was an unsaturated fatty acid, has a concentration of 39.2%.¹¹ Studies were showing that although palm oil was commonly referred to as palmitic oil was a saturated fat oil, palm oil behaves like unsaturated fatty oil, perhaps because of the content between saturated fatty acids and unsaturated fatty acids in this oil was almost balanced.¹²

The influence of palm oil in reducing total and LDL cholesterol levels also caused by the location of fatty acids on the glycerol backbone in palm oil. A study shows that the saturated fatty acids in palm oil esterified at

the *sn*-1,3 position rapidly digested by enzymes and delivered into the intestines, these fatty acids did not absorb efficiently, and most of them excreted in the feces. Meanwhile, unsaturated fatty acids esterified at the *sn*-2 position will well absorb as monoglycerides and transported to the liver, so that they can affect cholesterol homeostasis, just like oils that have high levels of unsaturated fatty acids such as oleic acid.^{13,14} In contrast to the total cholesterol, LDL, and HDL levels decreased after treatment, but triglyceride levels increased at all groups after treatment. There was an insignificant difference among all groups. However, what we can see that in each group there was an increase in triglyceride levels after treatment. That was in line with the significant increase in the body weight of rats in each group. Increased triglyceride levels were closely related to weight gain and central obesity.¹⁵ No previous research has looked at the effect of chicken oil on triglyceride levels, but there have been previous studies that showed an increase in triglyceride levels in mice given olive oil also had high oleic acid.¹⁶

Liver Histopathology

Although the examination of the serum lipid profile showed an advantage because it was able to reduce total and LDL cholesterol levels after being given chicken oil and palm oil after four weeks, histological examination of the rat liver showed steatosis in both groups. In this study, after four weeks of treatment, it turned out that in each group there was microvesicular steatosis of varying degrees, mild to moderate, and in each group, there were samples that had normal liver histology or had no steatosis (as shown in figure 2). However, in all groups, there was no macrovesicular steatosis.

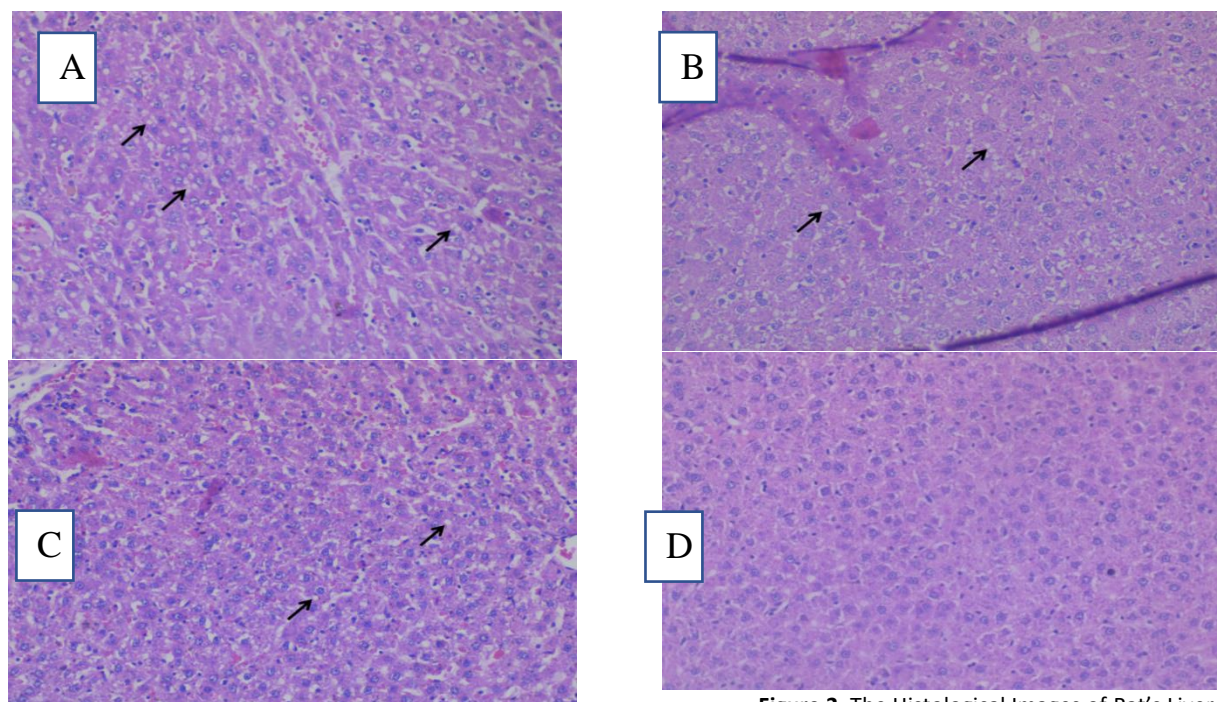


Figure 2. The Histological Images of Rat's Liver.

A).



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The rat's liver of group 1 treated with chicken oil shows moderate (++) microvesicular steatosis. B) The rat's liver of group 1 treated with chicken oil shows the presence of mild (+) microvesicular steatosis. C) The rat's liver of group 2 treated with palm oil shows mild (+) microvesicular steatosis. D) The rat's liver of group 2 treated palm oil did not show any steatosis. Black arrow: steatosis.

Data were processed statistically using the Mann-Whitney U test. The test results showed that the p -value = 0.419, which means an insignificant difference among groups.

Liver steatosis or fatty liver, mainly in non-alcoholic fatty liver disease (NAFLD) was intrahepatic triacylglycerol buildup of 5% or more by weight of the liver without adding factors such as heavy drinking or viral infection. There were several degrees of liver steatosis assessed based on the percentage of lipids in the hepatocytes: grade 0 (healthy, <5%), grade 1 (mild, 5% - 33%), grade 2 (moderate, 34% - 66%), and grade 3. (severe, > 66%).¹⁷

In this study, the steatosis formed was still mild to moderate microvesicular steatosis. Microvesicular steatosis was an accumulation of lipids in hepatocytes but has not caused a shift in the cell nucleus.¹⁸ Several things can cause liver steatosis, and one of them was a high-fat diet.¹⁹ Liver steatosis, especially in NAFLD, has a close relationship with triglyceride levels,²⁰ according to the results in this study after treatment in each group, there was an increase in triglycerides and the formation of microvesicular steatosis with varying degrees, mild to moderate.

Under normal conditions, there was a balance between uptake of plasma fatty acids by the liver and de novo lipogenesis, with the removal of lipids in the liver (FAO and VLDL). However, if an imbalance occurs, then steatosis will develop.¹⁹ The formation of microvesicular steatosis after the administration of chicken oil was still not fully understood, but a study shows that mice given a diet of olive oil rich in oleic acid can increase hepatic lipid levels. It was associated with decreased fatty acid oxidation in the liver.¹⁶ There was no significant difference between the steatosis that developed in the two groups and the steatosis that developed in mild to moderate degrees. However, it was unknown whether it was still not significantly different from palm oil if the chicken fat oil gave longer. So that more research needs on the effect of admitting chicken oil on health.

CONCLUSION

Consumption of 1 mL/day of chicken oil for four weeks had the similar effect on lipid profile and liver histology as consumption of 1 mL/day of palm oil with the same duration. Based on statistical tests, there is no significant difference between the two types of oil. The two oils lowered total cholesterol, LDL, and HDL levels, and raised triglyceride levels. Both also cause microvesicular steatosis but still mild to moderate degrees.

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