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Original Article

Feeding rats with used cooking oil elevates malondialdehyde, TNF-α, and creatinine compared to *tempe* fried with used oil

Retno Murwani[1,2], Neni Susilaningsih[2], Diaza O. Ariyanto[3*] and Ambariyanto Ambariyanto[1]

[1]Division of Natural Product Laboratory, Centre of Research and Services-Integrated Laboratory, Universitas Diponegoro, Semarang, Indonesia; [2]Department of Animal Science, Laboratory of Physiology and Biochemistry, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Semarang, Indonesia; [3]Department of Medicine, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

*Corresponding author: diazaokadimara@gmail.com

Abstract

In vivo studies on the hazards of deep-fried foods were commonly done by feeding used- or heated-cooking oil to rats. The aim of this study was to determine the effect of feeding *tempe* deep-fried in palm, olive, and coconut oils and the used frying oil on the blood biochemical profile of laboratory rats. An in vivo randomized control group study with pre-test and post-test was conducted. This study included healthy male Sprague-Dawley rats aged 2–3 months and weighing 100–200 grams. After acclimatization, the rats were randomly assigned to seven groups, which were: (1) regular diet (control diet); (2) diet of *tempe* deep-fried in 5× used palm oil (*Tempe*-in-used-Po); (3) diet of *tempe* deep-fried in 5× used coconut oil (*Tempe*-in-used-Co); (4) diet of *tempe* deep-fried in 5× used olive oil (*Tempe*-in-used-Oo); (5) diet of 5× used palm oil (Used-Po); (6) diet of 5× used coconut oil (Used-Co); and (7) diet of 5× used olive oil (Used-Oo). Each rat received 15 grams of a treatment diet daily and blood samples were collected after four weeks for a complete blood count and serum biochemistry analysis. The results showed that the final body weight and the weight gain of *Tempe*-in-used-Po, *Tempe*-in-used-Co, *Tempe*-in-used-Oo group, and Used-Po groups increased significantly compared to the control, Used-Co, and Used-Oo groups. However, there was a significant increase in serum tumor necrosis factor-alpha (TNF-α) in the Used-Co and Used-Oo groups ($p<0.05$), suggesting the used oil's detrimental effect. The Used-Co and Used-Oo were the only two groups whose creatinine increased significantly ($p<0.05$). Subsequently, only the Used-Oo group had a significantly increased malondialdehyde (MDA) level compared to all groups ($p<0.05$). These results prove that the effect of feeding fried food differs from used oils. Feeding used oil did not reflect the consumption of fried foods as part of the whole diet and generally resulted in more harmful effects. This is the first study to report an in vivo rat feeding study of deep-fried *tempe* and the used oil as part of the diet.

Keywords: Coconut oil, deep-frying, olive oil, palm oil, serum biochemistry

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Introduction

Deep frying is a popular and widely utilized cooking technique worldwide. It requires placing and submerging food into heated oil and rapidly cooking the inside while producing a crispy and golden-brown surface. Such texture and color make deep-fried foods one of the most favorite cuisines. There is a diverse range of deep-fried foods; for instance, french fries, chicken wings, and fish and chips are famous in many Western nations, whereas dumplings, tempura, deep-fried

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chicken and fish, and various chips are famous in Asian nations. The type of oil used for deep frying varies regionally as well. For instance, coconut and palm oil have been used for centuries in Asia, including Indonesia [1-3]. As globalization occurred, imported olive oil became popular and sold in many modern grocery stores around the world. Other vegetable cooking oils such as soybean, rapeseed, sunflower, corn, and peanut seed oil are used in different countries [4].

Tempe is a popular food made of fermented soybeans utilizing the fungus *Rhizopus oligosporus*. Although many studies have used the word *tempeh*, the original local name (Javanese language) is written and pronounced as tempe without "h" where the "e" is pronounced as light é instead of è. Historical records stated that it was a royal menu item in Java during the 17th century [5,6]. The fungal mycelia holds the soybeans together throughout the fermentation to form a dense cake-like structure [7]. During fermentation, the fungal enzymes break down complex proteins and carbohydrates in the soybeans, reducing soybean anti-nutrients such as phytate and anti-trypsin, resulting in more nutrient-rich *tempe*, which is easier to digest [8,9]. Fresh *tempe* contains 20.3% protein (59.6% moisture) [10], while ground-dried *tempe* contains 34.27% protein (5.47% moisture) [11]. Rich in minerals and fibers, *tempe* is an essential source of affordable, high-quality vegetable protein and serves as a main meal. It is one of the favorite foods consumed daily in Indonesia. It is estimated that 2.4 million metric tons of *tempe* are produced annually, with an average consumption of 6.5 kg per person yearly [12]. *Tempe* has been consumed also across the globe, including Japan, Suriname, the United States (US), the Netherlands, Canada, India, Guatemala, China, and Australia [6,13-16].

Fresh coconut oil has the highest percentage of saturated fatty acids, followed by palm and olive oil, which have the lowest percentage [17-19]. The highest monounsaturated fatty acid is in olive oil, followed by palm oil, and the lowest in coconut oil. Polyunsaturated fatty acid (PUFA) is also highest in olive oil, followed by palm oil, and coconut oil being the lowest [17-19]. Coconut oil is the most stable oil for deep frying *tempe* due to the highest percentage (91.15%) of saturated fatty acids [17]. Many studies examined the used cooking oil regarding the health risks of deep-fried foods or deep-frying hazards [20-23]. Numerous in vivo rat studies employed direct feeding of the used oil, not the fried food [24-28]. Directly feeding used cooking oil does not accurately reflect real-life consumption of fried foods, as people do not consume used cooking oil by itself. The cooking oil was absorbed by the fried foods during deep-frying and the fried foods are consumed along with the absorbed oil. Therefore, the aim of the study was to analyze the effect of *tempe* deep-fried in palm, coconut, and olive oils, as well as the used frying oils (palm, coconut, and olive oils) on the blood biochemical profile of laboratory rats.

Methods

Study design and animals

An in vivo study with pre-test and post-test was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia, in 2020. This study included healthy male Sprague-Dawley rats (*Rattus norvegicus*) aged 2–3 months and weighing 100–200 grams. The rats were obtained from PT. Bogor Labs, Bogor, Indonesia, and transported in an air-conditioned vehicle. They were housed in a cage with a wire-plastic roof for air circulation. During transport, the cage was furnished with bedding, food, and drinking water. Upon arrival at the Experimental Animal Laboratory, Faculty of Medicine, Universitas Diponegoro, the rats were housed randomly in four cages, each containing 20 rats. The acclimatization process involved keeping the rats in the laboratory at a room temperature of 25°C and 40±5% humidity, with 12-hour light/dark cycles for one week, providing regular feeding and *ad libitum* access to drinking water.

Study groups and intervention

After acclimatization, the rats were then weighed and 63 were allocated randomly to seven groups, consisting of nine rats in each. Randomization was performed by a computer according to the weight after acclimatization to ensure each group had a similar average body weight. The seven groups were: (1) regular diet (control diet); (2) diet of *tempe* deep-fried in 5× used palm oil (*Tempe*-in-used-Po); (3) diet of *tempe* deep-fried in 5× used coconut oil (*Tempe*-in-used-Co); (4)

diet of *tempe* deep-fried in 5× used olive oil (*Tempe*-in-used-Oo); (5) diet of 5× used palm oil (Used-Po); (6) diet of 5× used coconut oil (Used-Co); and (7) diet of 5× used olive oil (Used-Oo). Each rat from each group received a treatment diet of 15 grams per day with unlimited access to drinking water. The feed was prepared every morning and replaced the following day. After four weeks of treatment diets, blood samples were collected for a complete blood count and serum biochemistry analysis.

Deep-fried *tempe* preparation

Fresh *tempe* was cut into a rectangular shape to a typical household size of 3 cm × 9 cm × 1 cm. *Tempe* pieces were seasoned with salt and water and drained briefly before frying. An aluminum-based frying wok was used in the study. One liter of fresh oil was used to deep fry 20 pieces of *tempe*, ensuring all the *tempe* was submerged in the oil. The oil was poured into the wok and heated until ready to be used (130°C). *Tempe* pieces were placed into the hot oil and fried until golden brown. The initial oil temperature (130°C) for each oil (palm, coconut, and olive oil) and frying time (approximately seven minutes to reach golden brown) were recorded and used consistently for each frying repeat. Readily cooked *tempe* was taken off the oil and drained while the remaining oil was filtered and cooled for 30–45 minutes. After cooling, the remaining volume of oil was immediately used for the next frying repetition. Frying repeats of fresh *tempe* pieces were done five times with the same procedure. On the fifth repetition, the deep-fried *tempe* was collected, pooled, dried, and ground into a homogenous mesh for a diet containing deep-fried *tempe*. The homogenous fried *tempe* was used for rat diet formulation. Finally, the used oils were also gathered for diet preparation formulation.

Diet preparation containing deep-fried *tempe* and used oils

Complete feed for rats was formulated with reference to the AIN-93M diet for adult rats' maintenance [29] with some modifications. The composition was modified using locally available feed ingredients such as rice powder to replace corn starch to mimic the typical rice-based local diet as a primary carbohydrate source. For fat sources, instead of using soybean oil, palm oil was used (patent no. IDS000003805 and designated as a regular control rodent diet; 14% protein, 5% fiber, 4.4% fat). The *tempe* deep-fried 5× in the used oils was incorporated and mixed with other feed ingredients and formulated so that the proximate nutrient composition was the same as the control diet. In the diet of the fifth-used oil, the fifth-used oil replaced fresh palm oil to match the composition of the regular diet. The volume of oil used to substitute fresh palm oil as the constituent of the standard diet was calculated by a computer to mirror the detailed composition of the original standard diet precisely.

Measurement of weight before and after intervention

All rats were weighed before the intervention diet and after six weeks of intervention using EK-300i Compact Balance 300 g (A&D Medical, Michigan, USA) with 0.1 g sensitivity. The delta (Δ) weight of each rat was defined as the difference between the final weight and the initial weight.

Hematological and serum biochemical analysis

Blood samples were analyzed using the Prima Fully Auto Hematology Analyzer (OneLabMedika, Banten, Indonesia) at the Animal Health Laboratory, Semarang, Indonesia. Blood was drawn via the rat's jugular vein. Blood was directly placed in a vacutainer containing ethylenediaminetetraacetic acid (EDTA). Determination of red blood cells (RBC), hematocrit, white blood cells (WBC), platelets, MID cells (defined as eosinophils, basophils, monocytes, and precursors of WBC), and neutrophils was carried out by the electrification method, while hemoglobin was carried out by the cyanide-free hemoglobin spectrophotometry method. The red blood index included the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).

Serum samples were analyzed using the Indiko Clinical Chemistry Analyzer (Thermo Fischer Scientific, Waltham, USA) at the Inter-University Center (IUC) of Universitas Gadjah Mada, Yogyakarta, Indonesia. Briefly, blood was placed in a vacutainer without EDTA and the serum was separated by centrifugation at 4000 rpm for 10 minutes, collected in an Eppendorf tube, and

stored frozen until analysis [30,31]. The variables measured were total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, blood glucose, serum creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Measurement of serum tumor necrosis factor-alpha (TNF-α) and malondialdehyde (MDA)

Serum TNF-α and MDA were assessed using enzyme-linked immunosorbent assay (ELISA) kits, Rat Tumor Necrosis Factor A ELISA kit and Rat MDA ELISA kit, respectively (both from Shanghai Korain Biotech, Shanghai, China) with a Model 680 Microplate Reader (Bio-rad, Shanghai, China), following the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai, China). A total of 40 µL serum of each sample was also transferred into 96-well plates; afterwards, 10 µL anti-TNF-α antibody or anti-MDA antibody and 50 µL streptavidin-horseradish peroxidase (HRP) were added to the standard and sample wells. After incubation for one hour, the standard and sample wells were washed five times. In the next step, 50 µL of substrate solution A and 50 µL of substrate solution B were added to each well. They were incubated again for 10 minutes at 37°C in the dark. Finally, 50 µL of stop solution was added to each well and left to sit for another 30 minutes. The absorbance of samples in 96-well plates was measured in an ELISA reader at 450 nm wavelength.

Statistical analysis

The data were analyzed by ANOVA test to compare among groups, followed by post hoc (Duncan test) for normally distributed data or the Kruskal-Wallis test followed by Mann-Whitney for not-normally distributed data. For body weight, the difference between initial body weight and final body weight at the end of the experiment (Δ/delta) was analyzed with paired Student t-test or Wilcoxon test, as appropriate with data distribution. Statistical analyses were performed using SPSS version 16 for Windows (SPSS Inc., Chicago, USA). Statistical significance was considered at $p<0.05$.

Results

Weight measurement before and after intervention

Before the intervention, the rat body weight in each group was not significantly different, ensuring an equal baseline. After four weeks of the feeding experiment, there was a significant difference in the final body weight and weight gain (Δweight) across all groups ($p=0.007$). The highest final body weight was found in the *Tempe*-in-used-Co followed by *Tempe*-in-used-Po, *Tempe*-in-used-Oo, and Used-Po groups. The weight gain in the *Tempe*-in-used-Co ($p=0.018$), *Tempe*-in-used-Po ($p=0.008$), *Tempe*-in-used-Oo ($p=0.008$), and Used-Po ($p=0.012$) groups were higher than the control (**Table 1**).

Hematological analysis

There were no differences in most hematological indexes between groups apart from the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentrations (MCHC). Post-hoc analysis revealed that the MCH of the *Tempe*-in-used-Po group significantly increased compared to the Used-Po and Used-Co groups. For MCHC, only the Used-Po and Used-Co groups were significantly lower compared to the control group (**Table 2**).

Serum biochemical analysis

There were significant differences in serum TNF-α, MDA, and creatinine levels, with *p*-values of 0.007, 0.019, and 0.001, respectively. The post hoc analysis demonstrated that serum TNF-α was significantly higher ($p<0.05$) only in the Used-Po, Used-Co, and Used-Oo groups. Subsequently, only the Used-Oo group had a significantly higher MDA than all groups. Additionally, the Used-Co and Used-Oo are the only two groups whose creatinine was significantly higher ($p<0.05$) than the control group and the rest (**Table 3**).

Table 1. The mean weight of rats in each group before and after intervention

Variables	Control	*Tempe* and used palm oil	*Tempe* and used coconut oil	*Tempe* and used olive oil	Used palm oil	Used coconut oil	Used olive oil	*p*-value (between groups)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Initial weight (g)	121.89±13.8[a]	120.22±13.9	117.43±11.9	121.78±8.3	122.2±13.8	119.67±11.2	122.22±12.7	0.951[1]
Final weight (g)	164.78±9.2[a]	188.56±2.3[b]	190.00±2.6[c]	191.44±27.3[c]	184.6±14.5[b]	167.78±0.2[a]	171.78±26.6[a]	0.041[1*]
Δweight (g)	42.89±18.1[a]	68.33±14.0[b]	72.57±24.9[b]	69.67±27.8[b]	62.38±11.0[b]	48.11±14.0[a]	49.56±19.3[a]	0.007[1*]
p-value (between initial and final weight)	0.012[2*]	0.008[3*]	0.018[2*]	0.008[3*]	0.012[3*]	0.008[3*]	0.008[3*]	

Different letter superscripts (a, b, and c) within the same row mean significance at $p<0.05$
[1] Analyzed using Kruskall-Wallis test (if significant, followed by Mann-Whitney test)
[2] Analyzed using Mann-Whitney test
[3] Analyzed using Paired Student t-test
[*] Statistically significant at $p<0.05$

Table 2. Hematology parameters of each group after the intervention

Variables	Control	*Tempe* and used palm oil	*Tempe* and used coconut oil	*Tempe* and used olive oil	Used palm oil	Used coconut oil	Used olive oil	*p*-value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
White blood cells (10^3/µL)	14.26±2.5	18.28±2.7	16.24±3.0	16.43±5.9	21.11±9.0	16.70±6.0	19.39±6.5	0.242[1]
Red blood cells (10^6/µL)	5.87±0.4	6.04±0.4	6.10±0.5	5.92±0.5	5.91±0.8	6.19±0.2	6.27±0.4	0.534[1]
Hemoglobin (g/dL)	12.54±1.0	12.89±1.0	12.74±1.1	12.79±1.4	12.11±1.6	12.90±0.5	12.88±1.2	0.360[2]
Hematocrit (%)	53.58±4.3	54.60±3.6	54.57±3.1	58.62±13.5	52.71±6.9	57.70±2.6	54.96±4.5	0.352[1]
Platelet (10^3/µL)	708.11±80.0	690.11±138.2	705.10±77.7	809.00±121.5	687.50±118.1	738.00±28.3	722.60±71.2	0.157[1]
Lymphocyte (%)	10.18±2.2	13.50±2.5	10.83±3.7	11.39±4.4	14.53±5.3	11.64±4.3	13.74±5.6	0.272[1]
MID cells (%)	1.00±0.2	1.22±0.2	1.20±0.3	1.13±0.4	1.44±0.7	1.19±0.5	1.36±0.4	0.575[1]
Neutrophil (%)	3.18±0.6	3.56±0.9	4.21±2.4	3.91±1.3	5.15±4.0	3.90±1.4	4.29±1.0	0.397[1]
Mean corpuscular volume (µm^3)	91.41±4.5	83.36±23.8	91.29±3.3	92.79±3.0	89.41±2.4	89.70±9.9	87.71±3.8	0.076[1]
Mean corpuscular hemoglobin (pg)	23.37±0.7[a]	24.55±0.5[b]	23.29±1.1[a]	23.22±0.6[a]	22.90±0.4[c]	22.43±0.7[c]	23.30±0.5[a]	0.046[2*]
Mean corpuscular hemoglobin concentration (g/dL)	23.37±0.7[a]	23.50±0.5[a]	23.30±1.1[a]	23.30±0.6[a]	22.90±0.4[b]	22.40±0.7[b]	23.30±0.5[a]	0.013[1*]
Red cell distribution width, SD (µm^3)	36.90±6.5	35.90±3.6	36.89±9.5	35.70±4.4	35.30±3.1	37.16±6.6	33.22±3.3	0.798[1]
Red cell distribution width (%)	13.59±1.8	13.30±1.4	13.49±2.9	12.91±1.5	13.26±1.3	13.34±2.1	12.72±1.2	0.871[1]
Mean platelet volume (µm^3)	8.50±0.4	8.69±0.5	8.51±0.6	8.81±0.2	8.83±0.5	8.70±0.4	9.12±0.5	0.085[2]
Platelet distribution width (µm^3)	11.31±0.9	11.63±1.1	11.19±1.2	11.37±1.1	11.90±1.2	11.30±0.9	12.40±1.4	0.539[1]

Different letter superscript (a, b, and c) within the same row means significance at $p<0.05$
[1] Analyzed using Kruskal-Wallis test (if significant, followed by Mann-Whitney test)
[2] Analyzed using ANOVA test
[*] Statistically significant at $p<0.05$

Table 3. Serum biochemistry parameters of each group after the intervention

Variables	Control	*Tempe* and used palm oil	*Tempe* and used coconut oil	*Tempe* and used olive oil	Used palm oil	Used coconut oil	Used olive oil	*p*-value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Tumor necrosis factor-alpha (TNF-α) (pg/mL)	66.08±0.5[a]	66.19±0.6[a]	66.56±0.5[a]	66.60±1.6[a]	67.15±1.1[b]	67.06±0.7[b]	67.20±0.9[b]	0.007[1*]
MDA (nmol/mL)	0.73±0.1[a]	0.72±0.0[a]	0.73±0.0[a]	0.72±0.0[a]	0.72±0.0[a]	0.74±0.0[a]	0.75±0.0[b]	0.019[1*]
Cholesterol (mg/dL)	64.06±9.8	66.83±6.0	64.60±9.2	63.02±7.7	73.45±13.5	64.47±9.5	67.20±8.9	0.348[1]
High-density lipoprotein (mg/dL)	29.58±3.2	26.24±1.1	47.49±4.8	26.17±3.7	56.18±7.4	28.51±3.7	27.32±3.3	0.084[1]
Low-density lipoprotein (mg/dL)	12.91±4.7	12.87±2.3	13.63±3.6	13.56±3.8	15.64±5.7	12.37±2.9	15.16±3.6	0.552[2]
Triglyceride (mg/dL)	96.42±4.4[a]	68.56±3.7	92.97±1.7	91.28±3.5	95.41±3.1	74.93±0.1	84.52±1.7	0.578[2]
Glucose (mg/dL)	119.50±28.1	126.49±12.2	110.21±12.4	111.28±15.3	113.52±23.4	113.19±23.5	102.49±10.7	0.239[1]
Creatinine (mg/dL)	0.50±0.5[a]	0.46±0.1[a]	0.48±0.1[a]	0.50±0.1[a]	0.49±0.1[a]	0.57±0.04[b]	0.56±0.1[b]	0.001[2*]
Alanine aminotransferase (IU/L)	39.20±12.1	42.16±6.6	38.70±4.9	38.24±5.8	46.22±13.6	42.09±10.9	36.13±4.4	0.348[1]
Aspartate aminotransferase (IU/L)	98.47±25.8	97.12±27.4	95.31±10.8	88.89±20.0	106.70±30.4	87.82±15.3	90.00±20.4	0.618[1]

[1] Analyzed using Kruskal-Wallis test
[2] Analyzed using ANOVA test
[*] Statistical significance at $p<0.05$

Discussion

Different cooking oils demonstrated different effects on body weight. We observed that all diets containing fried *tempe* with used palm oil had higher average weight gain compared to the control group. These results align with a comparison study on Wistar rats which demonstrated a significant weight gain in combined consumption of regular food and fresh or used palm oil compared to sunflower oil [32]. Apart from weight changes, the study also showed alterations in the sizes of vital organs only in groups consuming reused oils. Another study incorporating oil as part of the diet (instead of direct oil consumption) showed that palm oil improved growth [33]. While the growth was accompanied by higher visceral fat accumulation, the hepatosomatic index, which represents tissue-specific lipid accumulation, did not increase. Adaptations to specific types of vegetable oils concluded that dietary vegetable oils had some effects on lipid metabolism without negatively affecting growth [33].

This study showed that only the used coconut and olive oils had lower average weight gain compared to the control group, while used palm oil had a higher impact on weight gain. Palm oil is indeed unique in this regard, in which many experiments demonstrated a positive effect on weight gain when compared with other types of oils despite the same base intervention [32-34]. Of the vegetable oils used in this study, olive oil contains the most monounsaturated fatty acid and polyunsaturated fatty acid [18,19]. Even though several animal studies concluded that a diet high in PUFA stimulated overweight [35-37], it does not directly translate to our study where there are diverse interactions between the deep-fried *tempe* and the cooking oil in terms of oil retention and changes in chemical properties [38] and deterioration of natural antioxidants. Even the cooking method plays a role, with an example of a study that evaluated the deterioration of natural antioxidants of vegetable oils showing a faster deterioration in pan-cooking compared to deep-frying [39].

It is important to note that studying the hazards of fried food through direct feeding of used oil or its components (e.g., fatty acids) is inadequate to reflect real-life situations such as evaluating weight gain. One of the reasons is that the cooking oils are absorbed by the food during frying, thus increasing its energy density and, to some degree, changing the food's chemical environment [38,40]. This aspect is absent in the methodology of direct feeding of used oil or its components. For example, a study of oleic acid supplementation in mice showed no change in weight gain [41]. Meanwhile, several previously mentioned studies have proven the positive effect on weight gain by consuming fried food with different oils [36,37].

In our study, the affected hematological parameters were MCH and MCHC, with significant differences in inflammation markers (TNF-α) and kidney function (creatinine). Determining whether the hematological parameters were abnormal was difficult. Data from the Health and Environmental Research Online database (HERO) of the United States Environmental Protection Agency (EPA) stated that for male rats aged 8–16 weeks old, the typical ranges of MCH and MCHC are 17.1–20.4 pg and 32.9–37.5 g/dL, respectively [42]. Another reference data from Korea stated that the typical ranges of MCH and MCHC in 13-week male Sprague-Dawley rats are 16.3–20.2 pg and 29.4–38.3 g/dL, respectively [43]. A more recent reference data from China for male Sprague-Dawley rats around five weeks of age determined that the typical ranges of MCH and MCHC are 18.70–21.20 pg and 31.0–33.6 g/dL, respectively [44]. From these ranges of values, we can assume that the levels in **Table 2** were high for MCH and low for MCHC when compared to the normal ranges. Regarding MCH, the values of used palm oil and coconut oil were significantly lower than those of the control group. Meanwhile, *tempe* deep-fried in used palm oil was significantly higher than in the control group. Regarding MCHC, only the used palm oil and used coconut oil groups were statistically lower than the control group.

We found that the serum biochemistry parameters with statistically significant differences were TNF-α, MDA, and creatinine. It is known that consuming a diet with thermally oxidized oil can elevate liver enzymes, cholesterol, and MDA [45]. Further development into non-alcoholic fatty liver disease is mediated by liver lipid peroxidation and the decrease of antioxidants, which seem to depend on the level of trans-fatty acids (TFA) in the oxidized oils [46]. The increased TFA in reheated oils may partly explain the cause of elevated TNF-α levels in only the used oil groups. TFA has been shown to induce inflammation and oxidative stress, marked by TNF-α [47]. Furthermore, several studies support the normal cholesterol levels observed in our study (**Table**

2), despite supposedly increased levels of TFA in used oils [48,49]. Even though TFA did not affect cholesterol levels in mice, it increased the risk of atherosclerotic diseases by directly harming the blood vessel walls [49].

Regarding MDA, only the used olive oil group had significantly higher levels compared to all other groups. MDA is formed during the last stage of the oxidation of PUFAs [50]. This occurrence can be explained by the higher content of PUFAs such as oleic, linoleic, and linolenic acid in olive oil when heated, compared to coconut oil and palm oil [51]. These differences in PUFAs did not appear prior to heating and after heating between palm oil and coconut oil. The used oil groups contrast with fried *tempe* groups because when *tempe* is fried at a temperature above 180°C, there is an increase in antioxidant level compared to fresh *tempe* [52]. The increase in antioxidants could neutralize the small amount of absorbed oil, preventing an increase in MDA and TNF-α levels [52].

Previous studies on rats that demonstrated an increase in creatinine levels used oral gavage as the method of oil delivery [53,54]. In our study, only the used coconut oil and used olive oil groups had an increase in creatinine levels compared to other groups. According to the fatty acid component in different heated oils, heated palm oil has the least amount of palmitic acid (C16:0) compared to heated olive oil and coconut oil, and the relationship is linearly increasing [51]. According to a study of molecular mediators of kidney injury in rats, saturated fatty acid palmitate upregulated the kidney injury molecule-1 (KIM-1), which is strongly correlated with damage to renal tubules [55], after which the rise of creatinine levels usually follows [56].

Conclusion

This study confirmed that the study of deep-fried *tempe* intake in rats differed from that of used oil. *Tempe* deep-fried 5× in used oils (palm, coconut, and olive oil) is not detrimental to rat physiology. However, a diet containing used oils affects rats' serum biochemistry differently, depending on their varied fatty acids constituent, with used palm oil having the least harmful effects. Further exploration is recommended on the long-term health impacts of consuming foods fried in repeatedly used oils and consider these findings when advising on cooking practices.

Ethics approval

The protocol of this study was approved by the Health Research Ethics Commission of the Faculty of Public Health, Diponegoro University, Semarang, Indonesia (No.106/EA/KEPK-FKM/2020).

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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