

COMPARATIVE STUDY OF OMEGA-3 EGG AND NON-OMEGA-3 EGG ADMINISTRATION ON MDA LEVELS IN MALE RATS (*Rattus norvegicus*) FED A HIGH-FAT DIET

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ABSTRACT

A high-fat diet is a major factor contributing to lipid metabolism disorders through increased synthesis and accumulation of cholesterol in the circulation. Hypercholesterolemia is closely associated with elevated oxidative stress, characterized by increased malondialdehyde (MDA) levels as a product of lipid peroxidation, thereby increasing the risk of cardiovascular disease. Omega-3-enriched eggs have been developed as functional foods containing omega-3 fatty acids with potential antioxidant properties that may reduce oxidative stress. This study aimed to determine the difference in blood malondialdehyde levels in high-fat diet-induced rats given omega-3 eggs compared to those given non-omega-3 eggs. This laboratory experimental study employed a post-test only with control group design using male *Rattus norvegicus* induced with a high-fat diet. The subjects were divided into a healthy control group, a high-fat diet control group, an omega-3 egg treatment group, and a non-omega-3 egg treatment group. After the intervention period, blood malondialdehyde levels were measured using the enzyme-linked immunosorbent assay (ELISA) method and analyzed using one-way ANOVA followed by Tukey's HSD post hoc test. The results showed a difference ($P < 0,05$) between the omega-3 egg group and the non-omega-3 egg group, indicating that omega-3 eggs exert a protective effect by attenuating the increase in MDA levels compared to non-omega-3 eggs. Administration of omega-3 eggs resulted in lower malondialdehyde levels compared to non-omega-3 eggs, suggesting that omega-3 content plays a role in suppressing the increase in blood malondialdehyde levels.

Keywords: High-Fat Diet, Malondialdehyde, Omega-3 Egg, Oxidative Stress

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INTRODUCTION

Consumption of a high-fat diet (HFD) is known to play an important role in the development of lipid metabolism disorders, particularly hypercholesterolemia, which is a major risk factor for cardiovascular disease and remains a leading cause of premature mortality both globally and nationally (Indriputri & Maulana, 2022; Isdadiyanto *et al.*, 2024; Perkeni, 2021). Hypercholesterolemia contributes to the development of atherosclerosis and increases morbidity and mortality, with the prevalence of abnormal cholesterol levels in Indonesia reaching 28.8% among individuals aged ≥ 15 years (Perkeni, 2021). Beyond its impact on cardiovascular disease, hypercholesterolemia also contributes substantially to the global burden of disability (Safitri *et al.*, 2023; WHF, 2019).

Hypercholesterolemia is closely associated with oxidative stress, a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense system. ROS promote lipid peroxidation, particularly of polyunsaturated fatty acids (PUFAs) in low-density lipoprotein (LDL), resulting in the formation of malondialdehyde (MDA), which is widely recognized as a stable and reliable biomarker of oxidative stress (Virdias *et al.*, 2024). Elevated MDA levels contribute to endothelial damage and LDL modification and are implicated in the pathogenesis of cardiovascular disease and DNA damage (Joneri *et al.*, 2024; Sulaiman & Sangging, 2024).

A diet high in saturated fat and cholesterol, including the consumption of animal-derived foods such as eggs, contributes to increased LDL cholesterol levels (Istiqomah *et al.*, 2024; Susanti *et al.*, 2024). Eggs are a widely consumed source of high-quality animal protein; however, their lipid and cholesterol content, particularly in the yolk, has raised concerns regarding excessive consumption (Lestari *et al.*, 2021; Ralahalu *et al.*, 2023). Omega-3-enriched eggs, produced through dietary modification in laying hens, have been developed to increase omega-3 fatty acid content while reducing the fat and cholesterol content of eggs (Soesanto *et al.*, 2023). Omega-3 fatty acids are known to exert hypolipidemic, anti-inflammatory, and antioxidant effects and may contribute to the reduction of oxidative stress and MDA levels (Rahima *et al.*, 2021; Mora *et al.*, 2023; Balachandar *et al.*, 2023; Astarini *et al.*, 2024).

Previous studies have demonstrated that omega-3 fatty acids and other sources of healthy fats can reduce MDA levels and improve lipid profiles in both experimental animals and humans (Shakoor *et al.*, 2020; Sari *et al.*, 2024; Suryo *et al.*, 2024). However, evidence regarding the effects of omega-3-enriched egg consumption on MDA levels, particularly under high-fat dietary conditions, remains limited. The novelty of the present study lies in the use of omega-3-enriched eggs as a functional food source and the direct evaluation of their effects on the oxidative stress biomarker MDA in an HFD-induced rat model. Therefore, this study aimed to evaluate the effect of omega-3-enriched egg administration on blood MDA levels in rats fed a high-fat diet. Although this study is expected to provide a scientific basis for the development of dietary strategies for the prevention of cardiovascular disease, its limitations, including the use of an animal model and the relatively short duration of HFD induction, should be considered. Further studies with longer intervention periods and human subjects are therefore warranted.

RESEARCH METHODS

This study employed a laboratory experimental design using a post-test-only control group design and a completely randomized design (CRD). A total of 22 male Wistar rats (*Rattus norvegicus*) were randomly allocated into four groups: the healthy control group (KS), high-fat diet (HFD) control group (KH), HFD + omega-3 egg group (P1), and HFD +

non-omega-3 egg group (P2). The experimental animals were acclimatized for 7 days before the intervention.

High-fat diet (HFD) induction was performed for 21 days using propylthiouracil (PTU) at a dose of 2 mg/200 g body weight/day, cholesterol at 0.2 g/200 g body weight/day, and duck egg yolk at 2.5 mL/200 g body weight/day. Subsequently, groups P1 and P2 received omega-3 eggs and non-omega-3 eggs, respectively, at a dose of 1.8 g/200 g body weight/day for 14 days. The sample size was calculated based on a minimum of four rats per group and adjusted for a potential 10% dropout rate, resulting in a required sample size of 5–7 rats per group. Based on this calculation, 24 rats were initially included in the study to account for potential dropouts. The study population consisted of male Wistar rats (*Rattus norvegicus*) approximately 2 months of age and weighing 180–200 g. The samples were selected based on predetermined inclusion and exclusion criteria, with consideration of potential dropouts. The experimental animals were acclimatized for 7 days before the intervention. The data were obtained from measurements of total cholesterol and serum malondialdehyde (MDA) levels in the rats following the intervention.

The main equipment used in this study included an electronic balance, oral gavage tube, micropipettes and tips, centrifuge, spectrophotometer, 96-well microplate, and ELISA reader. The materials consisted of male Wistar rats (*Rattus norvegicus*), Comfeed AD II standard laboratory feed, omega-3 eggs and non-omega-3 eggs (*PT Eryfarm Berkah Mahadana, Indonesia*), duck egg yolk, propylthiouracil (PTU), cholesterol powder, an MDA assay kit (*Bioassay Technology Laboratory, Shanghai, China*), and distilled water. The study commenced with the submission and approval of ethical clearance, followed by a 7-day acclimatization period for the experimental animals. HFD induction was performed for 21 days in groups KH, P1, and P2 through oral administration of PTU, cholesterol powder, and duck egg yolk using an oral gavage tube. The success of HFD induction was evaluated by measuring total cholesterol levels using the cholesterol oxidase–phenol aminophenazone (CHOD-PAP) method.

Following the induction period, group P1 received omega-3 eggs, while group P2 received non-omega-3 eggs at a dose of 1.8 g/200 g body weight/day (equivalent to 1.75 mL/200 g body weight/day) for 14 days, whereas the control groups received standard feed. On day 42, blood samples were collected from the retro-orbital plexus following an 8-hour fasting period. Serum samples were subsequently used to determine MDA levels using the sandwich *enzyme-linked immunosorbent assay* (ELISA) method. The study commenced following approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Soedirman (ethical clearance number: 073/KEPK/PE/VI/2025). The experimental animals were subsequently acclimatized for 7 days. HFD induction was performed for 21 days in groups KH, P1, and P2 using PTU at a dose of 2 mg/200 g body weight/day, cholesterol powder at 0.2 g/200 g body weight/day, and duck egg yolk at 2.5 mL/200 g body weight/day, administered orally using an oral gavage tube.

The success of HFD induction was evaluated based on changes in total cholesterol levels after 21 days of HFD administration compared with those in the healthy control group. Total cholesterol levels were measured using the cholesterol oxidase–phenol aminophenazone (CHOD-PAP) method. Following the induction period, group P1 received omega-3 eggs, while group P2 received non-omega-3 eggs at a dose of 1.8 g/200 g body weight/day (equivalent to 1.75 mL/200 g body weight/day) for 14 days, whereas the control

groups received standard feed. On day 42, blood samples were collected from the retro-orbital plexus following an 8-hour fasting period. Serum samples were subsequently used to determine MDA levels using the sandwich enzyme-linked immunosorbent assay (ELISA) method.

RESULTS AND DISCUSSION

Results

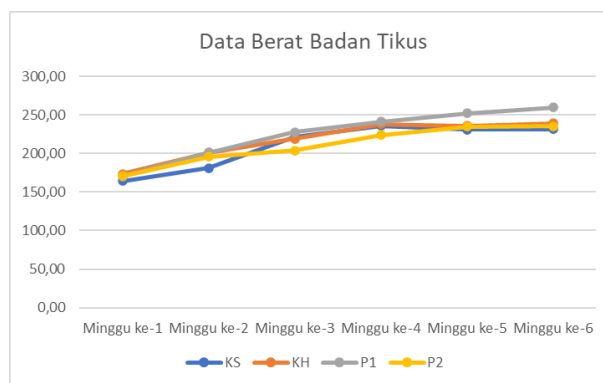


Figure 1. Rat Body Weight Graph

As shown in Figure 4.1, all rat groups experienced an increase in body weight during the initial induction phase. An increase from the first to the second week was observed in groups KS, KH, P1, and P2. From the second to the fourth week, the most pronounced increase was observed in group KS, whereas groups KH and P1 showed relatively stable increases, and group P2 showed no substantial change. Subsequently, from the fourth to the sixth week, body weight increased again in groups KH, P1, and P2, while group KS experienced a decrease.

Table 1. Univariate Analysis of MDA Levels (nmol/mL)

Group	n	Mean±SD
KS	7	1,947±0,389
KH	5	2,025±0,168
P1	4	2,410±0,270
P2	6	2,775±0,277

Note: KS: Healthy control group; KH: HFD control group; P1: HFD induction + omega-3 egg at 1.8 g/200 g body weight; P2: HFD induction + non-omega-3 egg at 1.8 g/200 g body weight; SD: standard deviation.

The univariate analysis showed that the highest mean MDA level was observed in group P2 (2.775 nmol/mL), followed by groups P1, KH, and KS, with mean values of 2.410, 2.025, and 1.947 nmol/mL, respectively. The highest standard deviation was observed in group KS (± 0.389), followed by groups P1, P2, and KH, with standard deviations of ± 0.277 , ± 0.277 , and ± 0.168 , respectively.

Table 2. Bivariate Analysis of MDA Levels (nmol/mL)

Group	Mean $\pm$ SD	Shapiro–Wilk Test Significance	Levene’s Test Significance	One-Way ANOVA Significance
KS	1,95±0,39	0,311	0,224	<0,001*

KH	2,03±0,17	0,283
P1	2,41±0,27	0,483
P2	2,78±0,28	0,418

Note: KS: Healthy control group; KH: HFD control group; P1: HFD induction + omega-3 egg at 1.8 g/200 g body weight; P2: HFD induction + non-omega-3 egg at 1.8 g/200 g body weight; SD: standard deviation.

Bivariate analysis of MDA levels was performed using one-way analysis of variance (ANOVA) because the data met the assumptions of normality ($p > 0.05$) and homogeneity of variance ($p > 0.05$). The analysis yielded a significance value of $p < 0.001$ ($p < 0.05$), indicating a statistically significant difference in MDA levels among the treatment groups. Therefore, the analysis was continued with the Tukey HSD post hoc test.

Table 3. Results of the Tukey HSD Post Hoc Test

Group	Group	<i>p-value</i>	Interpretation
KS	KH	0,970	Not significant
	P1	0,101	Not significant
	P2	<0,001	Significant
KH	KS	0,970	Not significant
	P1	0,259	Not significant
	P2	0,003	Significant
P1	KS	0,101	Not significant
	KH	0,259	Not significant
	P2	0,269	Not significant
P2	KS	<0,001	Significant
	KH	0,003	Significant
	P1	0,269	Not significant

Note: KS: Healthy control group; KH: HFD control group; P1: HFD induction + omega-3 egg at 1.8 g/200 g body weight; P2: HFD induction + non-omega-3 egg at 1.8 g/200 g body weight; SD: standard deviation.

The Tukey HSD post hoc test showed a statistically significant difference in MDA levels between the healthy control group (KS) and the non-omega-3 egg group receiving 1.8 g/200 g body weight (P2), with a p -value of <0.001 ($p < 0.05$). A statistically significant difference was also observed between the HFD control group (KH) and the P2 group, with a p -value of 0.003 ($p < 0.05$).

Discussion

This laboratory experimental study employed a post-test-only control group design to evaluate the effect of omega-3 egg administration on malondialdehyde (MDA) levels in male Wistar rats (*Rattus norvegicus*) induced with a high-fat diet (HFD). The study was

conducted over 42 days. A total of 24 rats were initially included to account for potential dropouts, of which 22 completed the study and were included in the analysis. The rats were divided into four groups: the healthy control group (KS), HFD control group (KH), HFD + omega-3 egg group (P1), and HFD + non-omega-3 egg group (P2). The characteristics of the experimental animals showed changes in body weight throughout the study. The KH, P1, and P2 groups receiving HFD exhibited relatively consistent increases in body weight, whereas the healthy control group showed less pronounced fluctuations. The pattern of weight gain observed in the HFD groups may reflect a physiological response to a high-fat diet, consistent with the findings of Campos *et al.* (2025), who reported that HFD contributes to increased body weight in rats through adipose tissue accumulation.

MDA levels, as a biomarker of oxidative stress, differed significantly among the groups based on one-way analysis of variance (ANOVA) ($p < 0.001$). The P2 group (non-omega-3 egg) exhibited the highest mean MDA level, followed by P1, KH, and KS. The Tukey HSD post hoc analysis showed significant differences between P2 and both KS and KH, whereas the differences between P1 and the other groups were not statistically significant. Descriptively, the P2 group had a higher MDA level than P1. However, the difference in MDA levels between P1 and P2 was not statistically significant ($p = 0.269$); therefore, this study did not demonstrate a statistically significant difference in MDA levels between rats receiving omega-3 eggs and those receiving non-omega-3 eggs.

Biologically, elevated MDA levels under HFD conditions may be associated with increased production of reactive oxygen species (ROS) resulting from excessive lipid metabolism. ROS promote lipid peroxidation of polyunsaturated fatty acids, generating MDA as an end product. This mechanism has been widely reported, with HFD being associated with increased oxidative stress and inflammatory pathway activation through elevated ROS production and nuclear factor kappa B (NF- κ B) activation (Tan & Norhaizan, 2019; Shabalala *et al.*, 2022).

Descriptively, the omega-3 egg group (P1) exhibited lower MDA levels than the non-omega-3 egg group (P2), although the difference was not statistically significant ($p = 0.269$). This descriptive difference may theoretically be associated with the omega-3 fatty acid content, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have anti-inflammatory and antioxidant effects through mechanisms involving suppression of NADPH oxidase activity and enhancement of endogenous antioxidant defenses (Bayram & Kiziltan, 2024). However, MDA levels in P1 remained higher than those in the control groups and therefore did not descriptively indicate a reduction to control levels. Furthermore, these mechanisms cannot be confirmed by the present study because the bioactive components of the eggs were not directly measured. In addition, the multiple double bonds in EPA and DHA make these fatty acids susceptible to oxidation, which may theoretically influence oxidative stress responses, as reported by Rudia *et al.* (2023) and Yassir *et al.* (2023).

Conversely, the P2 group exhibited the highest MDA levels descriptively. This difference may theoretically be associated with differences in the fatty acid composition of omega-3 and non-omega-3 eggs. High cholesterol content may theoretically impair mitochondrial function, reduce mitochondrial glutathione levels, and increase ROS production, thereby promoting lipid peroxidation and MDA formation (Rauchbach *et al.*, 2022). This finding is consistent with previous studies reporting an association between high cholesterol intake and increased oxidative stress biomarkers. However, the present study did not analyze the nutrient composition or the cholesterol and omega-3 content of the eggs administered. Therefore, the association between egg composition and MDA levels in the P2 group cannot be established based on the present findings.

Overall, the omega-3 egg group exhibited a lower mean MDA level than the non-omega-3 egg group; however, this difference was not statistically significant ($p = 0.269$). These findings indicate a descriptive difference but do not provide sufficient evidence to demonstrate an effect of omega-3 egg administration on MDA levels. The potential role of omega-3 eggs as a functional food in modulating oxidative stress warrants further investigation, particularly through studies with longer induction and intervention periods and direct analysis of egg composition. The limitations of this study include the relatively short duration of HFD induction and the composition of the high-fat diet, which may have been insufficient to induce marked hypercholesterolemia. In addition, cholesterol and MDA levels were not measured before HFD induction. These limitations should be considered when interpreting the findings and provide a basis for future studies employing longer intervention periods and more comprehensive study designs.

CONCLUSION

Administration of omega-3 eggs to rats fed a high-fat diet resulted in descriptively lower MDA levels compared with non-omega-3 eggs, although the difference was not statistically significant ($p = 0.269$). These findings do not demonstrate a significant difference in MDA levels between omega-3 and non-omega-3 egg administration. Therefore, the potential of omega-3 eggs as a functional food for modulating oxidative stress cannot be determined based on MDA levels alone. Nevertheless, these descriptive findings may provide a basis for future studies involving longer HFD induction periods, measurement of biomarkers before and after intervention, and the use of multiple oxidative stress biomarkers to provide a more comprehensive assessment.

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