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Effect of Different Types of High-Fat Diet (HFD) for Induction of Type 2 Diabetes **Mellitus in Animal Model**

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Abstract

Consumption of fast food increase the risk of type 2 diabetes mellitus (T2DM) through excessive fat accumulation in adipose tissue, insulin resistance, and βcell dysfuntion. Studies comparing different fat types for T2DM induction are scarce. This study aims to identify the optimal fat type for T2DM induction in animal model, based on cholesterol increase rate and blood glucose stability. The induction of T2DM was performed using high-fat diet (HFD) for 8 weeks and alloxan intraperitoneally. The HFD consist of 80% standard feed, 15% fat sources (palm oil, beef tallow, or goat tallow), and 5% duck egg yolk. This study used a completely randomized design with 20 male DDY mice grouped into five treatment groups. T2DM was assessed based on fasting blood glucose levels and islet diameter of Langerhans in mice. Data of fasting blood glucose levels were analyzed using ANOVA and Duncan's test. Data on islet diameter were analyzed using Kruskal-Wallis and Dunnett's test. The results showed that different fat types had a significant effect (p<0.05) in induced T2DM. Among the three types of fat, beef tallow was the most optimal for inducing T2DM according to its ability to increase cholesterol levels and stability in maintaining blood glucose levels.

Keywords:

blood glucose levels; high-fat diet; saturated fat; diabetes mellitus

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INTRODUCTION

Excessive and continuous in consumption of fast food has become a new trend in society. Based on Chen et al. (2023), fast food is often high in calories but low in fiber. Frequent consumption of these foods contributes to excessive caloric intake and obesity, which are major risk factors for type 2 diabetes mellitus (T2DM). This condition is caused by excess fat accumulation that can leading to insulin resistance (Jusuf et al., 2024). Consequently, impairment Glucose Transporter Type 4 (GLUT4) signaling pathways due to insulin resistance leads to high glucose levels/hyperglycemia (Fakar et al., 2023).

Obesity is defined as a condition where excess fat accumulates in the body, which has the potentially dangerous to health (WHO, 2023). Patients are not only adults, but also teenagers and children. At least 3.7 million people in worldwide die every year due to obesity (Xie et al., 2025). Hariyani & Riani (2024) stated that obesity in Indonesia affects 20% of school-age children, 14% of teenagers, and 35.5% of adults. Obesity occurs due to excessive fast food consumption. The saturated fat content in fast food can increase Low-density lipoprotein (LDL), lower High-density lipotrotein (HDL), and insulin resistance (Pipoyan et al., 2021). Hasneli et al. (2024) stated that high LDL increases the risk of hipercholesterol and triglyceride levels in the blood. Hypercholesterolemia is diagnosed when blood cholesterol level exceeds 200 mg/dL (PERKENI, 2019a).

In the body, fat is converted into free fatty acids and stored as triglycerides in adipose tissue (Telisa et al., 2020). Excess fat causes adipose tissue hypertrophy, which ultimately disrupts the insulin signaling pathway (Handari et al., 2023). The maximum daily fat intake for adults is 46 g, or approximately 20-35% (Kole et al., 2020, USDA & HHS, 2020). Novela (2019) stated 1g of fat produces 9kcal, which is more than carbohydrates and protein. Fat in fast food can be sourced from plant based or animal fats.

Palm oil is a plant-based fat widely used in fast food. This type of oil is often chosen because it imparts a savory flavor to food and leaves little residual oil (Yani & Irawan, 2019). Palm oil consists of 50% saturated fat, 40% monounsaturated fat, and 10% polyunsaturated fat (Hasneli et al., 2024). In





addition to plant-based fats, a high-fat diet can also be caused by excessive consumption of animal fats. Examples of animal fats include beef and goat tallow. Forouhi *et al.* (2018) stated the main fat content in beef and goat are saturated fatty acids. Beef tallow has a saturated fat content of 17.68 g, while goat tallow has 11.1 g (Mustofa & Putri, 2022). The World Health Organization (WHO) recommends consumption of saturated fat to be less than 10% of total daily calories, or 20 g for women and 30 g for men (WHO, 2023; Mozaffarian *et al.*, 2018).

Excessive consumption of saturated fat in fast food can lead to various dangerous diseases, such as obesity and T2DM (Janah & Lastariwati, 2016). Obesity can lead to T2DM through insulin resistance. This is due to elevated levels of free fatty acids (saturated fat) in adipose tissue impairs fatty acid oxidation and interfere with insulin pathway in regulating glucose uptake (Sandika, 2020). The decreased ability of mitochondria to oxidize fat also leads to the accumulation of lipotoxic metabolites such as diacylglycerol and ceramide (Murru *et al.*, 2022). These metabolites activate Protein Kinase C (PKC), thereby inhibiting tyrosine phosphorylation of the Insulin Receptor Substrate (IRS) (Sánchez-Alegría & Arias, 2023).

Patients are diagnosed with diabetes mellitus (DM) when random blood glucose levels is \geq 200 mg/dL, blood glucose level from an oral glucose tolerance test (OGTT) is \geq 200 mg/dL, and fasting blood glucose (FBG) level is \geq 126 mg/dL (PERKENI, 2019b; Lestari *et al.*, 2021). Unstable blood glucose levels in T2DM is resulted from insulin resistance and pancreatic β cell dysfunction. This condition is also caused by increased oxidative stress in the islets of Langerhans, where β cells produce insulin (Leenders *et al.*, 2021). Insulin resistance occurs when insulin sensitive tissues show a decreased response to insulin signals, thus inhibiting glucose uptake (Bingga, 2021). This impaired ability in glucose uptake forces pancreatic β cells to secrete excess insulin, ultimately leading to cell damage (Ardika *et al.*, 2024). International Diabetes Federation (IDF) reports that the number of diabetes mellitus patients worldwide in 2024 will be 589 million people (ages 20-79) with more than 90% of them having type 2 diabetes, while in Southeast Asia, approximately 106.9 million people or 10.8% are recorded (IDF, 2025). Type 2 diabetes mellitus (T2DM) in obese individuals occurs because of impaired metabolism. This condition reduces insulin receptor sensitivity as a result of excess fat accumulation (Handari *et al.*, 2023). The high prevalence of T2DM in obese individuals has led to multiple studies using animal models of diabetes.

Husna $et\ al.\ (2019)$ stated that animal models in diabetes research should ideally exhibit phenotypes or characteristics that reflect the pathogenesis of DM as it occurs in humans. Animal models are considered optimal for T2DM models if their cholesterol levels are elevated to hypercholesterolemia within a short period, maintain consistent hyperglycemia after alloxan induction, and induced pancreatic β cell damage, as indicated by a reduced diameter of the islets of Langerhans (Akinlade $et\ al.$, 2021). Feeding a high-fat diet (HFD) in animal models aims to increase cholesterol levels, and is usually followed by alloxan or streptozotocin (STZ) induction for establishing hyperglycemia, thereby representing type 2 diabetes mellitus (T2DM) conditions (Skovsø, 2014). Lin $et\ al.\ (2023)$ reported that the treatment group fed a high-fat diet showed increased cholesterol levels. Similarly, Oktaviona & Qomariyah (2023) demonstrated that HFD using palm oil induced obesity in mice and elevated both free fatty acids and blood glucose levels. According to Putu $et\ al.\ (2020)$, the combination of HFD and alloxan induction in mice can induce more stable hyperglycemia and better represent T2DM condition.

However, studies comparing different types of fat to determine which type of fat in HFD is optimal for T2DM induction have not been conducted in depth. Therefore, further research is needed to identify the type of fat in HFD that is optimal for inducing T2DM in animal models. The novelty of this study lies in comparing different fat types in HFD to determine the optimal fat for inducing T2DM in animal models, based on their ability to increase cholesterol levels, maintain stable blood glucose levels, and impair pancreatic β -cell function. This provides a reference for future studies and informs the public about which fat type is most closely associated with type 2 diabetes.

MATERIALS AND METHODS

This research was an experimental study using a completely randomized design (CRD) consisting of a control group (standard feed (KK)), a placebo group (standard feed + 5% duck egg yolk + alloxan monohydrate (KP), and three HFD treatment groups (palm oil (K1), beef (K2), or goat tallow (K3) followed with alloxan monohydrate). Feeding, either HFD or standard feed, was carried out for 8 weeks before induction with alloxan monohydrate. Each treatment group included four replicates. This research was conducted from January to May 2025 at the Laboratory of Experimental Animals, Animal Physiology, and Biological Microtechnics, Universitas Negeri Surabaya.



All groups of mice were fed according to the treatment group for 8 weeks ad libitum. The control group (KK) mice were only given standard feed. The placebo group (KP) mice were given standard feed + 5% duck egg yolk. Meanwhile, the HFD treatment group was given high-fat feed. All group were also given water ad libtum. The high-fat feed consisted of 80% of standard feed, 15% fat, and 5% duck egg yolk (Tatto *et al.*, 2017). Variations in the type of fat used were solid palm oil, goat tallow, or beef tallow heated in a pan to produce oil. The high-fat feed was made by grinding 400 grams of standard Comfeed BR1 using a blender, then mixing it with 25 grams of duck egg yolk and 75 grams of various types of oils (palm oil, goat tallow, or beef tallow). After mixing, the high-fat feed was placed on a tray and dried in an oven at 60°C for 3 days.

This study used male mice of the Deutsch Denken Yoken (DDY) strain aged 6–8 weeks, weighing between 25–30 g, with a total of 20 individuals. The mice were acclimated for 7 days in cages 46×30×12 cm and provided with wire covers and rice husks as bedding. During the acclimation period, all mice were given standard Comfeed BR1 and water ad libitum. After acclimation, each mice was given feed according to the treatment group for 8 weeks. After 8 weeks or when the mice reached hypercholesterolemia (>130 mg/dL), the mice were induced with a 100 mg/kg BW dose of alloxan monohydrate intraperitoneally (Zhang *et al.*, 2016), except for the control group (KK). On the 3rd day after the injection, the fasting blood glucose (FBG) levels of all groups were measured to determine whether the mice experienced hyperglycemia (FBG >126 mg/dL) (Setiadi *et al.*, 2020).

Fasting blood glucose (FBG) levels were measured six times: before the HFD was administered, post-HFD (day 0/after 8 weeks of feed administration followed by alloxan induction), and on days 3, 7, 14, and 21 following the administration of alloxan monohydrate. According to Putri *et al.* (2017), the mice were fasted for 12 hours prior to having their fasting blood glucose levels measured. An *EasyTouch GCU* glucometer and blood glucose test strips were used to check FBG level from peripheral blood. Measurements from day 7 to day 21 were used to assess the stability of blood glucose levels, consistent with previous studies reporting stable glucose readings in mice during this period (Noge *et al.*, 2025). Mice were declared diabetic when FBG >126 mg/dL (PERKENI, 2019).

The preparation of pancreatic histopathology specimens was carried out on the 23rd day after alloxan induction. Mice were anesthetized and dissected. Pancreas was removed and washed with physiological NaCl, then soaked in 10% neutral buffered formalin solution overnight. The tissue was subsequently rinsed under flowing tap water. Pancreas was then soaked in 70% alcohol (4 times), 80% (2 times), and 96%, followed by absolute alcohol for 30 minutes at each concentration. The process continued to the clearing stage by soaking the pancreatic tissue in xylene 1 for 15 minutes, then in xylene 2 overnight. Next, the infiltration process was carried out by soaking the tissue in a xylene:paraffin solution (1:1) for 30 minutes, then soaking it in pure paraffin three times, each for 1 hour. Liquid paraffin was then poured into the base mold, and the pancreatic tissue was placed inside and left to harden overnight. Tissue block was cut with a rotary microtome at 4 µm thickness. After being placed on a water bath at 40°C, the tissue sections were moved to a glass slide and put in oven for two hours. Pancreas sections were then stained with Hematoxylin Eosin (HE) staining (Khaleyla *et al.*, 2021).

The Langerhans islets in mice were observed histologically under a light microscope. The diameter of the Langerhans islets was measured using ImageJ 1.53 (Rahmania, 2020). As shown in Figure 1, a calculation formula based on Fadhilah (2021) research and a measurement sketch based on Sasmita et al. (2024) were used to determine the average diameter of the Langerhans islets, as the following.

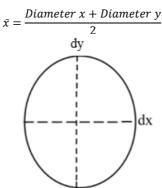


Figure 1. Measurement of Langerhans islet diameter (μ m). Diameter x= horizontal diameter, Diameter y= vertical diameter.



The data obtained in this study were FBG levels (mg/dL) and the diameter of the islets of Langerhans (μ m). Fasting blood glucose data were analyzed using one-way ANOVA and post-hoc Duncan test. Meanwhile, the islets of Langerhans data were analyzed using Kruskal-Wallis test followed by Dunnett test.

RESULTS

Based on the results of the research that has been conducted, the fasting blood glucose levels from all treatment groups are presented in Table 1. Blood glucose data were collected on the pre-HFD day, post-HFD (after HFD administration followed by alloxan induction), and on days 3, 7, 14, and 21 after alloxan induction. Fasting blood glucose levels in all treatment groups during pre-HFD and post-HFD remained within the normal range (<126 mg/dL). However, an increase in fasting blood glucose levels was observed at post-HFD (H0) compared to pre-HFD, with post-HFD values approaching the diagnostic threshold for DM.

Tabel 1. Mean fasting blood glucose levels of mice in each treatment group

Treatment	Fasting Blood Glucose Levels of Mice (mg/dL)					
Group	Pre-HFD	Post-HFD	Day-3	Day-7	Day-14	Day-21
KK	90.75 ±	103.50 ±	102.75 ±	104.25 ±	111.00 ±	115.50 ±
	13.92a	8.38a	9.09a	6.42a	3.00^{a}	3.84^{a}
KP	103.25 ±	113.00 ±	129.00 ±	137.25 ±	134.50 ±	131.25 ±
	16.53a	9.00a	2.24 ^b	7.53 ^b	4.97^{b}	5.72 ^b
K1	85.00 ±	117.75 ±	143.75 ±	150.75 ±	141.00 ±	141.75 ±
	20.33a	10.26a	9.96^{bc}	9.63b	14.87^{b}	8.84^{b}
K2	107.75 ±	116.00 ±	183.25 ±	181.25 ±	176.00 ±	180.25 ±
	5.31a	10.12a	13.03 ^d	13.14 ^c	8.49^{c}	8.01 ^c
К3	86.00 ±	110.50 ±	147.25 ±	151.75 ±	142.50 ±	143.75 ±
	4.12^{a}	8.79a	7.79^{c}	12.17 ^b	14.79^{b}	12.50 ^b

Notes: Different superscript notations indicate significant different (p<0.05) in each measurement based on Dunnett,s test. KK= Control Group (standard feed, without HFD and alloxan), KP= Placebo Group (Standard feed + alloxan + 5% duck egg yolk), K1= HFD with Palm Oil Group (plant-based fat HFD + alloxan), K2= HFD with Beef Tallow Group (beef tallow HFD + alloxan), K3= HFD with Goat Tallow Group (goat tallow HFD + alloxan).

The administration of a high-fat diet (HFD) to a group of mice for 8 weeks aimed to induce hypercholesterolemia and diabetes mellitus conditions. The average cholesterol levels after 8 weeks of HFD administration in the treatment groups of mice showed that the highest cholesterol level was 132.00 ± 4.24 mg/dL in the beef tallow group (K2). The goat tallow group (K3) had a cholesterol level of 117.50 ± 7.14 mg/dL, and the palm oil group (K1) had a cholesterol level of 116.25 ± 5.12 mg/dL. In the control group (KK), after 8 weeks of standard feed administration, the cholesterol level was 112.25 ± 4.82 mg/dL, and in the placebo group (KP), after 8 weeks of standard feed + 5% duck egg yolk, the cholesterol level was 107.00 ± 3.37 mg/dL.

Mice in the KP, K1, K2, and K3 groups experienced an increase in fasting blood glucose levels that exceeded the normal range (>126 mg/dL) after receiving group-specific HFD and alloxan induction. Meanwhile, the control group (KK), which received only standard feed without alloxan induction, consistently had fasting blood glucose levels below 126 mg/dL. On day 21 after alloxan induction, the treatment group fed with the HFD beef tallow diet (K2) maintained persistently elevated fasting blood glucose levels, reaching 180.25 ± 8.01 mg/dL.

The results of statistical analysis on FBG levels of mice on day 21 after alloxan induction demonstrated that the data were statistically significant (p = 0.000; p<0.05). These findings indicate that HFD feeding with palm oil, beef tallow, or goat tallow combined with alloxan induction elevated fasting blood glucose levels above the diagnostic threshold for diabetes mellitus (>126 mg/dL) and maintained them within the hyperglycaemic range. In addition, the administration of standard feed with 5% duck egg yolk (KP group) for 8 weeks also increased fasting blood glucose levels above the diagnostic threshold for diabetes mellitus (>126 mg/dL) and sustained them within the hyperglycaemic range on day 21 after alloxan induction, but the increase was not as high as in the three HFD fat groups.

The quantitative data on the diameter of the islets of Langerhans in each treatment group of mice are presented in Table 2, while the histological observations of the islets of Langerhans are shown in Figure 2. According to Table 2, the diameter of the islets of Langerhans in the treatment groups fed with HFD and alloxan (K1, K2, and K3) was smaller than that in the control group (KK). The KP group



also showed a smaller diameter compared with the KK group, which was attributed to alloxan induction. The mean diameters of the islets of Langerhans were 599.95 \pm 190.23 μ m in the KK group, 523.59 \pm 161.22 μ m in the KP group, 356.47 \pm 108.71 μ m in the K1 group, 199.63 \pm 107.26 μ m in the K2 group, and 276.66 \pm 132.50 μ m in the K3 group. The result indicated that the smallest diameter was observed in the beef tallow (K2), while the largest diameter was found in the control group (KK).

Group given beef tallow HFD group (K2) was significantly different from the KK, KP, and K1 groups, but not significantly different from the K3 group.

Table 2. Mean Langerhans islet diameter in each treatment group

Treatment Group	Langerhans Islet Diameter (μm)		
KK	599.95 ± 190.23a		
KP	523.59 ± 161.22a		
K1	356.47 ± 108.71 ^b		
K2	199.63 ± 107.26 ^c		
K3	276.66 ± 132.50bc		

Notes: Different superscript notations indicate significant different (p<0.05) in each measurement based on Dunnett,s test. KK= Control Group (standard feed, without HFD and alloxan), KP= Placebo Group (Standard feed + alloxan + 5% duck egg yolk), K1= HFD with Palm Oil Group (plant-based fat HFD + alloxan), K2= HFD with Beef Tallow Group (beef tallow HFD + alloxan), K3= HFD with Goat Tallow Group (goat tallow HFD + alloxan).

The histopathological sections of the Langerhans islets in mice is presented in Figure 2. Microscopic observations showed that the pancreas consists of acinar cells, which surround the islet cells. The Langerhans islets are located within the pancreatic tissue among these acinar cells. In the control group (KK), both the acinar cells and the islet cells appeared to have a denser and more compact tissue structure.

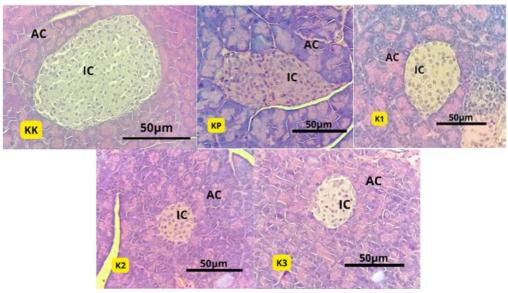


Figure 2. Histology of Islets of Langerhans in Type 2 DM Mice. Notes: KK= Control Group, KP= Placebo Group, K1 = HFD with Palm Oil Group, K2= HFD with Beef Tallow Group, K3= HFD with Goat Tallow Group. IC= Islet cell, AC= Asinar cell.

DISCUSSION

Excessive intake of high-fat diets (HFD), particularly from fast food, is widely recognized as a major contributor to obesity and type 2 diabetes mellitus (T2DM). The presence of high levels of saturated fats and calories increases circulating triglycerides and free fatty acids, which in turn promote adipose tissue hypertrophy and insulin resistance (Paleva, 2019). In this study, HFD was applied to elevate cholesterol levels, thereby simulating an obesity-related metabolic state. Alloxan was subsequently administered to induce β -cell damage, mimicking the dual pathogenesis of T2DM (Skovsø, 2014).

Obesity caused by the accumulation of free fatty acids disrupts the body's metabolic response to insulin signaling, thereby increasing the risk of T2DM. Insulin resistance occurs when insulinsensitive tissues (receptor cells) fail to respond to insulin signals, thus impairing the glucose uptake



process (Bingga, 2021). This occurs because free fatty acids activate PKC and inhibitor kinase B, which inhibit tyrosine phosphorylation of the insulin receptor (Vargas *et al.*, 2019). As a result, IRS fails to activate Phosphatidylinositol 3-kinase (PI3K), resulting in downregulation of GLUT4 translocation, leading to lower glucose uptake into the cells (Sánchez-Alegría and Arias, 2023).

Alloxan following HFD induces T2DM either by generating reactive oxygen species (ROS), which trigger oxidative stress, or by inhibiting glucokinase activity, thereby blocking insulin secretion (Wulandari *et al.*, 2024). According to Sindi *et al.* (2022), the increase in ROS damages pancreatic β -cell DNA, preventing these cells from secreting insulin. When β cells fail to release insulin, hyperglycemia worsens, leading to elevated blood glucose levels. Based on fasting blood glucose (FBG) measurements conducted from day 7 to day 21 after alloxan induction, the FBG levels in all treatment groups of mice remained relatively stable at \geq 126 mg/dL, except in the control group (KK). These findings indicate that following HFD feeding and alloxan monohydrate induction, the mice were in a hyperglycemic state consistent with T2DM.

Rahardjo *et al.* (2021) reported that fats derived from animals, such as goat or beef, contain saturated fatty acids that are more likely to cause degenerative diseases than vegetable fats. Similarly, Utama *et al.* (2024) demonstrated that beef fat increases blood glucose levels compared to palm oil and corn oil due to its higher content of free fatty acids and LDL cholesterol. Furthermore, Shi *et al.* (2022) showed that cholesterol levels are positively associated with blood glucose levels in the development of hyperglycemia in mice.

In the 8 weeks of HFD feeding, the highest cholesterol level was recorded in the K2 group mice, which was 132.00 ± 4.24 mg/dL, while the KK, KP, K1, and K3 groups had cholesterol levels in: 112.25 ± 4.82 mg/dL; 107.00 ± 3.37 mg/dL; 116.25 ± 5.12 mg/dL; 117.75 ± 7.14 mg/dL. In the placebo group (KP) that was given standard feed and 5% duck egg yolk, the FBG levels on the 21-day after alloxan administration remained stable above the normal threshold at 131.25 ± 5.72 mg/dL. However, the group couldn't be categorized as a T2DM models because the cholesterol levels after 8 weeks of feeding were still below hypercholesterolemia range (<130 mg/dL). This also applied to the palm kernel oil solid group (K1) and the goat tallow group (K3). The characteristics of T2DM refer to the study by Gheibi *et al.* (2017), which indicates that insulin resistance occurs, leading to β -cell dysfunction in the pancreas similar to that in humans due to excessive fat consumption.

The decrease in the diameter of the Langerhans islets in T2DM mice can occur due to high levels of ROS, which cause oxidative stress and dysfunction in pancreatic β cells (Wulandari *et al.*, 2024). In this study, the reduction in the diameter of the Langerhans islets occureds, among other factors, due to exposure to diabetogenic agents such as alloxan, which induces oxidative stress and generates free radicals (Santoso *et al.*, 2024). Research by Walean *et al.* (2020) explains that the pancreas of mice inducted with alloxan will experience atrophy, decreasing the size of Langerhans islets because the β cells are forced to produce more insulin on top of oxidative stress (Ardika *et al.*, 2024). The damage to the β cells reduces insulin production, causing hyperglycaemia (Setadi *et al.*, 2020).

In addition to the administration of diabetogenic substances, the reduction in the diameter of the Langerhans islets in this study is also caused by the increase in free fatty acids resulting from the high-fat diet (HFD). Wali *et al.* (2020) reported that the accumulation of saturated fat in adipose tissue induces inflammation and oxidative stress. This accumulation further impairs β -cell function and disrupts glucose homeostasis. Since β cells are highly susceptible to oxidative stress, the elevation of ROS exacerbates cellular damage and reduces insulin secretion (Mukai *et al.*, 2022). According to Raza *et al.* (2019), exposure to alloxan and palmitic acid significantly increases ROS production and decreases the activity of glutathione reductase and other antioxidant enzymes. Excessive ROS disrupts the redox balance and causes damage to cell membranes, mitochondria, and β -cell DNA (Juan *et al.*, 2021). Over time, damage to these components leads to β -cell death through either apoptosis or necrosis.

The average diameter of the islets of Langerhans in the KK group was the largest at 599.95 \pm 190.23 μm , followed by the KP group with an average diameter of the islets of Langerhans of 523.59 \pm 161.22 μm . Then, in the three treatment groups, the average diameter of the islets of Langerhans was decreased, at 356.47 \pm 108.71 μm in the K1 group; 199.63 \pm 107.26 μm in the K2 group and 276.66 \pm 132.50 μm in the K3 group. However, of the three treatment groups, it was known that mice fed with beef tallow (K2) experienced the highest decrease in the diameter of the islets of Langerhans.

CONCLUSION

Beef tallow in the HFD (K2 group) was found to be optimal in inducing type 2 DM in the mice (*Mus musculus*) model, as indicated by a rapid elevation of cholesterol levels, fasting blood glucose



levels that remained stable after alloxan monohydrate administration, and the smallest diameter of the islets of Langerhans among all treatment groups.

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CONFLICT OF INTEREST

There is no conflict of interest.

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