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The Effect Of Traditional And Industrial Nusa Tenggara Timur Smoked Curing "Se'i" On Levels LDL (Low Density Lipoprotein) Cholesterol In Experimental Animals (Study On Male Wistar Rats Induced With Pork Fat)

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Abstract

Background : The prevalence of central obesity in Kupang City is higher than the prevalence in the province, which is 10.29%.It gets along with the escalation of pork se'i consumption which is estimated by Association of Farmer and Pork Entrepreneur that pork se'i production in some outlets in Kupang can reach 2,5 ton per day or more than 500 porks per day. The differences in physicochemical characteristics of industrial pig se'i and domestic pig se'i along with the potential for an increase in LDL cholesterol in individuals caused by the saturated fat content in pork se'i need to be observed in vivo.

Objective : This study was to determine differences in LDL cholesterol in experimental animals before and after administration of pork se'i.

Methods: This research is a true experimental with a pre-test and post-test controlled design was conducted using male wistar mice with weight 150-160 gram and age 8-12 weeks. The pork se'i was mixed with water with 1:17 concentration and given to the mice through oral sonde. The intervention was last for 30 days with daily intervention to the mice. Research subjects were divided into five groups, namely the positive control group, negative control group, and treatment at a dose of 0.89 gram/day; 1.78 grams/day; and 3.56 grams/day. The negative control group was given standard feed and

drink ad libitum while the positive control group was induced using HFD (High Fat Diet) consisting of corn oil (80%), lard (5%), and chicken egg yolk (15%).

Results **:** The average difference in LDL cholesterol levels after and before the intervention in all treatment groups was -9.44 mg/dl with the difference in average LDL cholesterol levels after and before the intervention being the highest in the control group. Independent T-Test analysis found that there was a significant difference between the group given a high-fat diet and the group given 1.78 grams of industrial

pork se'i in one day marked with a significance result of 0.047 <0.05. The other group did not show any significance which was marked by a significance result of more than 0.05.

Conclusion : The study was that there was no relationship between the consumption of industrial pork se'i and an increase in LDL cholesterol in experimental animals.

Keywords**:** Pork Se'i, Low Density Lipoprotein, Traditional and Industrial.

Introduction

Smoked curing se'i pork produced by Kupang, Indonesia. It is a popular product in Nusa Tenggara Timur (NTT) since it has a particular smell, color, and flavor (1). Se'i that processed by NTT society traditionally used to use kitchen salt, sendawa chili, red meat, and kusambi leaf (Schleichera oleosa) as source of the smoke so that the smoke can keep the color of the meat to stay reddish or bright reddish (2). Traditionally processed meat usually has 2-3 cm for each cut and it is usually mixed with salt and potassium nitrate(KNO3) for draining as the next step. The addition of nitrate in pork se'i processing is proposed to inhibit the pathogenic microbe such as Clostridium botulinum from growing and developing which cause rancid in pork sei product (3). The purpose of adding KNO3/KNO2 in pork se'i is because nutrient content for 100 grams of pork sei is 32 grams of protein and 63% water which makes pork sei easily contaminated by microbes so that it has a shorter shelf life (4). The traditional process of making pork se'i doesn't use KNO3/KNO2 as a food preservative, but those produced by industry have food preservatives in the form of KNO2 and KNO3.

Unfortunately, the addition of KNO3 and KNO2 in pork has a bad effect on the health of the consumer. KNO3 and KNO2 which are added into the pork are proposed to enhance the color and flavor of the meat so that it is more pleasant to be seen and eaten, which the main precursor of N-nitroso (5). Nitrosation endogenous is contributing to around 45-75% of total human exposure to N-nitroso compound (NOC) (6) Previous study conducted in retired person in America shows that the incidence of Renal Cell Carcinoma (RCC) is increasing among those population whose consumption of nitrite coming from animal meat is in highest quantile. The hazard ratio (HR) also shows that those who consume highest nitrite coming from animal meat is 1,28 bigger than those who consume lowest nitrate coming from animal meat (7). This study also concluded that patients with highest consumption of nitrate and nitrite coming from processed meat will have a higher risk of experiencing RCC(HR=1,17) compared to those who consume lower amounts of nitrate and nitrite (7). Similar study with cohort retrospective methods also shows that there is a significant association between RCC and hamburger and sausage consumption with adjusted Odd Ratio for highest level versus lowest as much as 1,4 (8). Even nitrate and nitrite also can be found in vegetables and fruits but the study shows that there is a significant inverse association between increasing consumption of vegetables and vegetable juice both for male and female with Renal Cell Carcinoma (8). The inverse association shows that the higher vegetable and vegetable juice consumption the lower risk of Renal Cell Carcinoma (8). The cause of RCC in retired people associated with red meat consumption is usually linked to the other components of the meat such as heme iron, HCAs, PAHs, nitrite, and nitrate (9).

Previous study conducted in America for Retired Person (NIH-AARP) is a cohort retrospective study that shows there is an association between nitrate and nitrite consumption coming from processed meat with increasing risk of Renal Cell Carcinoma (RCC) (7). Nitrate contained in the processed meat and pork se'i can be reduced to nitrite by the flora and bacteria both in the mouth or other part of the digestive tract. In the acidic gastric, nitrite will form nitrosating agents that react with secondary amines or alkylamides so that NOCs will occur. Those amines and alkyl amides are unfortunately found in the animal based product especially those processed ones that can trigger tumors of the kidney in animal based study (10) Amines and amides compounds found in the animal based products are expected to lead to a greater endogenous NOC production (11). N-nitroso or NOC (Nitroso compound) is one of carcinogens which is easily produced through interaction from secondary amino with nitrosation triggering agents which usually exist in nitrite salt

(12). Foods which are treated with nitrate salt to increase color, flavor, and preservation are suspected to be a triggering agent of nitrosation (12). Nitrate salt is one of the ingredients produced by industry. There is 500 mg of nitrate salt in every 1 kg of pork for pork se'i production (13).

N-nitroso compound is one of carcinogens that can induce tumors or cancer in several targets such as rats. The cancer or tumor occurrence is usually influenced by its dosage, frequency of the intervention, and route of the intervention. N-nitroso compounds are proven to have effect on several organs such as kidney and liver along with some cases there is a change from hepatocyte to endothelial cell after N-nitroso exposure (14). Not only in preclinical study, intake of nitrosamine as the precursor of N-nitroso compounds from food among people is also examined in Hong Kong and other coastal Tiongkok cities. It is tempting to see that the high incidence of nasopharyngeal cancer in these societies is associated with the consumption of nitrosamines which are usually contained in the smoked fish cured by nitrite salt (14).

As the effect of N-nitroso compounds in several organs including kidney along with the data indicating increasing consumption of red meat especially pork se'i, the information related to association between pork se'i and kidney function problem needs to be deeply explained. With the high fatality rate of RCC proven by the statistical data showing that 50% of RCC patients have a bad prognosis (15). Neoplasm growth in RCC patients is dependent on its proliferation and death rate of the cancer cell. p53 is one of the genes which suppress tumorigenesis located in the 17p chromosome which specifically implicates the control of checkpoint during G1 phase of cell cycle by monitoring DNA state before entering S phase (16). The damaged DNA and cell usually will experience apoptosis due to the blockage of G1 phase in the cell cycle. If there is a mutation, the p53 gene will be inactivated and lose its heterozygosity ability continued by the apoptosis inhibition and cause tumor progression (17). The existence of p53 mutation in some tumors usually associated with alteration of the genetic in human cancer and usually accumulated in nucleus which is immunohistochemically detectable (18). The inhibition of p53 will lead to the impair function of apoptosis, namely programmed cell death as a negative regulating system in the growth of neoplasm (19). One of the indications of impaired apoptosis is the over expression of Bcl2 which inhibits apoptosis and contributes to the development of tumors and modifies their clinical behavior. Multiple studies have proven that there is an association between Bcl2 overexpression and many carcinomas such as breast, lung, ovary, bladder, and prostate (20). This p53 and Bcl-2 expression can be a prudent clinical marker to investigate the prognosis of renal cell carcinoma. The study conducted by Ali K, et al

(2005) also declared that p53 and Bcl2 are important prognostic factors in RCC tumors (21). This study shows that the incidence of p53 mutation is 35% which reflects that p53 mutations are not common in RCC, but it still has a significant prognostic (20).

Unfortunately, the previous study doesn't seem to explain the exact clinical markers of the Renal Cell Carcinoma, so this study will elaborate more about the protein expression abnormality which can trigger Renal Cell Carcinoma. Previous study conducted by Hu et al (2003) also mentioned that the limitation of retrospective cohort study that they conducted is the study can not investigate association of nitrate and nitrite by food source due to the large, prospective design, and detailed assessment (8). Previous study also examined the effect of nitrate salt curing in fish which contain less amine and amide compared to red meat. Further research conducted in red meat based products cured using nitrate salt should be conducted to see the effect on human health especially important organs such as the kidney. Especially the effect on the mutation of protein expressions such as p53 and Bcl2. Research shows that p53 overexpression demonstrates association with grade, stage, and tumor diameter even if it is not associated with age, gender, side of disease and cellular pattern (21). P53 is still a very prudent clinical marker for RCC prognosis due to the 83,3% of 5-year survival rate in patients with non-staining tumors vs 46,6% for patients with p53 positive staining tumors (22). Meanwhile, Bcl2 also can be used as a clinical marker for cancer cells which is shown by the immunoreactivity of Bcl-2 detected mainly in the cytoplasm and nuclei of cancer cells and has expressed in 64% of the cancer cases (19).

Objective : This study was to determine differences in LDL cholesterol in experimental animals before and after administration of pork se'i.

Matterials and Methods

Materials

The types of pork se'i given to the experimental animals were pork se'i with a higher saturated fat content (industrial pork se'i) made from landrace pork and se'i with low LDL content (traditional pork) made from NTT village pork.

Industrial pork se'i is pork sei produced on a large scale, where the roaster is made of iron, packaged by the vacuum method, and the content of food additives is lower. Traditional pork sei is pork se'i produced on a large scale, where the grill is made of kosambi wood, without a vacuum, and does not contain preservatives.

Methods

This research is a true experimental with a pre-test and post-test controlled design was conducted using male wistar mice with weight 150-160 gram and age 8-12 weeks. This study studied the relationship between industrial pig sei and home hog sei with the content of saturated fatty acids in the form of myristic acid (C14:0) and palmitic fatty acid (C16:0), and cholesterol which affects LDL cholesterol levels in experimental animals.

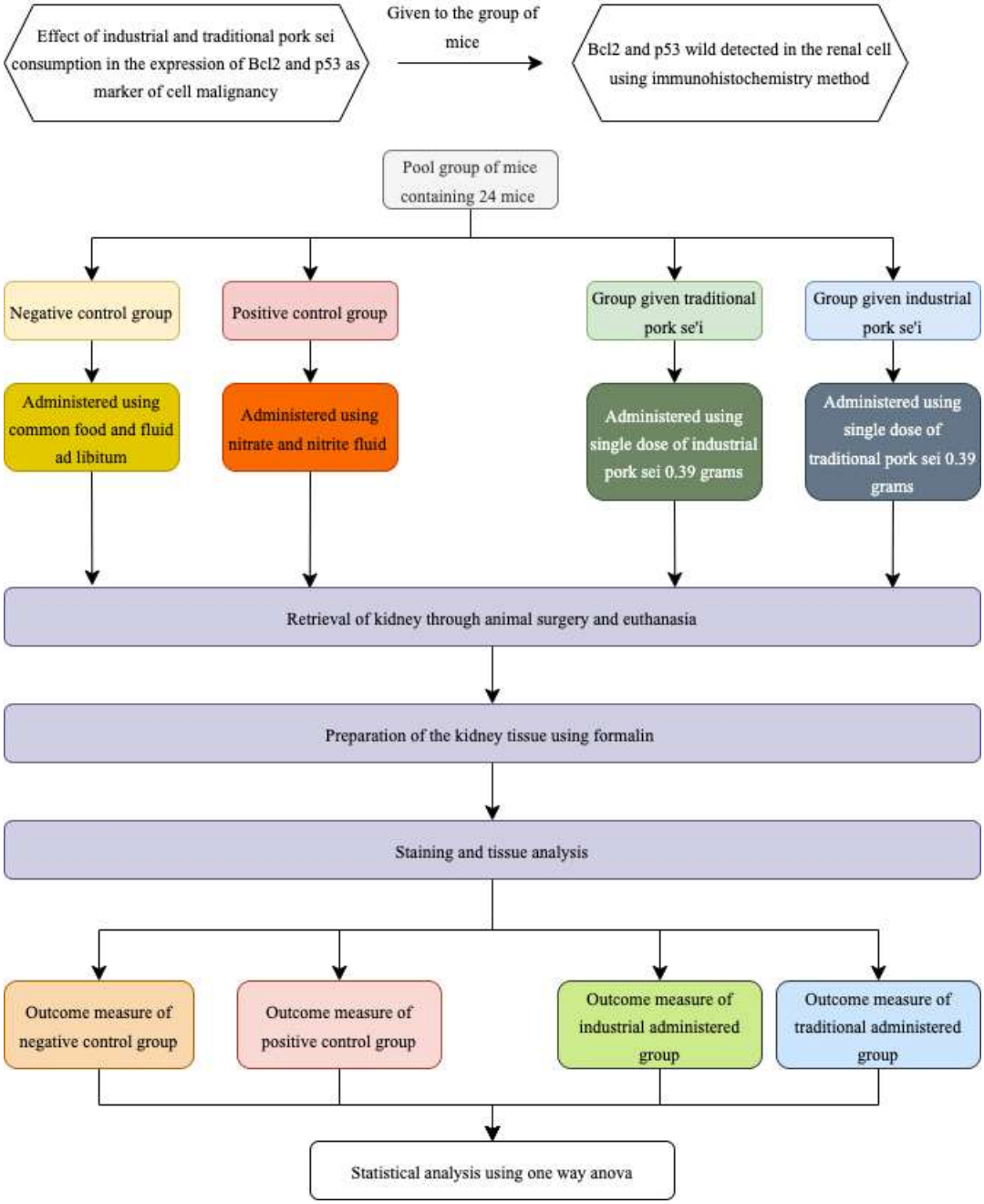

Figure 1. Study plan of industrial and traditional pork sei towards Bcl2 and p53 expression.

This study was conduct an experimental study and completely randomized design consists of 2-arm parallel groups consisting of an intervention group and a control group. Intervention groups are given pork sei which are produced by large scale industry that use KNO2 and KNO3 as food preservative, meanwhile the control group is given the home made pork sei that use KNO2 and KNO3 in greater amounts. A one month intervention to the male wistar is going to be conducted in the Laboratory of Biochemistry Universitas Airlangga.

Based on figure 1, this research was initiated by intervening mice's daily meal using pork sei. Nitrate and nitrite in pork sei will alter the apoptosis in the renal cell and cause Bcl2 and p53 expression abnormalities. There will be 24 mice as a pool group which was separated into four groups containing 6 mice of each. The first group will be given the industrial pork sei containing a higher number of salpeter salt of nitrate and nitrite as much as 0.39 grams in form of liquid and given to the intervention group orally. And the second group will be intervened using traditional pork sei which contains a lower concentration of nitrate and nitrite. Beside the intervention group intervene using traditional pork se'i and industrial pork se'I, there was a negative control group which will receive common food for mouse along with its normal fluid via ad libitum. The positive control group will be intervene using fluid containing nitrate and nitrite to induce malignancy. After being intervened for 30 days, the mice will be euthanized using ether and then the kidney will be retrieved using standardized surgical method. Before analyzing the p53 and bcl2 expression in the mice kidney the sample will be prepared and stained so that the tissue can be analyzed. From the intervention and analysis this research will have two outcomes named as outcome 1 for industrial pork sei intervention and outcome two for traditional pork sei intervention. After gaining the result from both groups then the data will be statistically analyzed using a one way anova statistical method. This study has been ethically approved by Faculty of Public Health University of Airlangga ethical committee in number 185/EA/KEPK/2022.

Sample Size

Wistar needed for the research is counted using Federer Formula which is determined based on the number of groups as mentioned below (Federer):

$(t-1)(n-1) \geq 15$

$(5-1)(n-1) \geq 15$

$4n - 4 \geq 15$

$4n \geq 19$

$N \geq 4.75 \sim 5$

From the formula above, we can conclude that the number of wistar in each group is 5. So that the minimum number of wistar is 24. Specification of the wistar is 150-160 grams in weight, with age 8-12 weeks. The dosage of each intervention for pork se'i is counted using formula of conversion from 70 kg human. The formula of dosage counting is mentioned below:

The daily consumption of pork se'i in Kupang is 100 grams for every 5 people. It can be concluded that each person consumes 20 grams of pork se'i on their daily basis. The conversion of dosage from 70 kg human to 25 grams mouse is 0,0026, so that the formula can be broken down into:

Dosage for 25 grams mouse = 20 gram x 0,0026
= 0.052 gram

Dosage for 150 grams mouse = 0.052 gram x (150 gram/20 gram)
= 52 mg x 7.5
= 390 mg
= 0.39 gram

So the dosage for each mouse with weight 150 grams 0.39 g for each kind of the pork se'i. The maximum capacity of mouse gastric is 3 ml, so that we can give 2 ml of pork se'i solution to the mice by mixing it with water. For 0.39 g pork se'i was mixed with 1.61 ml water. The solution was 0.195 M or represented as every 1 ml of water will contain 0.195 gram of pork se'i.

Sample size of the intervention is 32 mice with two groups with two intervention containing intervention in form of traditional pork sei and industrial pork sei as mentioned in Table.1

Table 1. Group Separation by Intervention

Groups	Treatment	Number of Mice
1	Negative Control Group	5
2	Positive Control Group	5
3	Dose 0.89 gr pork se'i	5
4	Dose 1,78 gr pork se'i	5
5	Dose 3,56 gr pork se'i	5

Inclusion and Exclusion Criteria

Inclusion criteria for each groups are the wistar should be classified as Rattus norvegicus strain wistar, male, with minimum age 8 weeks and maximum age 12 weeks, each wistar has to be around 150-160 grams in weight confirmed with weight measurement using digital scale before the research executed, and the wistar included in the research should be healthy. Healthy wistar indicated by the active movement, thick fur, clear eyes, and able to eat well.

Exclusion criteria for each group are sick wistar before the research indicated by the passive movement, avoidance to eat, dull and easy to fall hair. Mentally stressed wistar also can not be used in the research indicated by changes of the behavior related to the decrease of active movement, avoidance to drink, frequently lick the body of itself, and being more aggressive and loud (23).

Randomization

The animals were randomized after surviving the initial, using a computer based random order generator. The wistar was acclimatized for 7 days, after that every wistar was given a number from 1-24. Those number was inserted into the random pick generator in the computer and separated to a different cage. Every cage was labelled based on the intervention given to the related group of wistar.

Data Collection Procedures

Maintenance of Experimental Animals

Rats adapted for 7 days in the experimental laboratory. Wistar rats are kept in cages that are equipped with places to eat and drink. Cage cleaning is done every 2 days by applying the 3R and 5F principles.

Preparation of Industrial Pork Se'i Solution and Traditional Pork Se'i

The average household consumption/week in Kupang is 4 kg. The number of family members in one family is 3-5 people. Each person consumes 0.114.3 kg of se'i every day or about 114.3 grams. **Group 1** : The weight of the pork given = 0.0026 x 114.3 gram = 0.297 gram/kg BW of the wistar rat Weight of pork se'i for one wistar rat = 0.297 gram x 150 gram/25 gram; = 1.782 grams of pork sei ~ 1.78 gram. **Group 2** : The weight of the pork given = 0.0026 x 57.15 gram = 0.149 gram/kg BW of the wistar rat Weight of se'i pork for one wistar rat = 0.149 gram x 150 gram/25 gram, = 3.564 grams of pork sei ~ 3.56 grams. **Group 3** : The weight of the pork given = 0.0026 x 228.6 gram = 0.594 gram/kg BW of wistar rats Weight of

pork se'i for one wistar rat = 0.594 gram x 150 gram/25 gram, = 0.894 gram pork sei ~ 0.89 gram.
The process of consuming pork se'I was given by sonde per oral using a 40 cm terumo tube with a full liquid food texture which will be given to rats as much as 5 ml. The volume given adjusts to the stomach capacity of rats, which is 1 ml/20 grams or 7.5 ml in rats weighing 150 gram.

Preparation of LDL Cholesterol Induction Solution
The composition of the saturated fat induction food is a mixture of 80% corn oil, 15% lard, and 5% duck egg yolk. This composition showed a significant increase in cholesterol and total triglyceride levels in experimental animals.

Experimental Animal Treatment
Wistar was weighed again to determine his weight before being induced. It is using markers and plastic. Next, separate the positive, negative, and treatment groups into control groups.

Administration of Industrial Pork Se'i Solution and Traditional Pork Se'i
Wistar is held with the left hand, on the shoulder with the thumb in front, the palm is placed on the back. Four other fingers are circled on the abdomen to hold the wistar while administering the pork se'i solution which will be given through a tube. The part of the wistar's stomach should not be pressed to prevent the wistar from vomiting during the administration of the pork se'i solution. Food is given slowly through a tube into the mouth (±5 cm).

Blood Sampling Process of Experimental Animals
Wistar was anesthetized with ketamine injection of 50-150 mg/kg weight. The laboratory assistant takes a 3 ml insulin syringe and injects it at an angle of 15 degrees on the wistar tail. Blood was transferred into an EDTA tube. Blood is stored in a cool box and given ice gel to prevent blood lysis. Maximum limit of blood taken through the vein on the wistar tail is 1.5 ml. This is to prevent
wistar's death.

Process of LDL Analysis
The steps are: Take 100 µl of serum then add 1000 µl of LDL precipitating reagent; Homogenization using a vortex; Centrifugation for 15 minutes at 4000 rpm; Mixing serum that has been centrifuged using 100 µl supernatant solution; Add 1000 µl of LDL kit enzyme reagent then homogenize using a vortex; Incubate the sample for 10 minutes at 20-

25^{o}C; Reading levels with a clinical spectrophotometer in this case LDL-cholesterol levels (mg/dl) = total cholesterol (mg/dl) - cholesterol in the supernatant (mg/dl).

Data Analysis

Parametric data, were analyzed using Analysis of Variance (ANOVA) followed by a multiple range test. Non-parametric data were analyzed using the Kruskal-Wallis test followed by Mann-Whitney test. All analyses were performed using SPSS software version 24.

RESULTS AND DISCUSSION

1. **Physio-chemical Properties**

The data of physio-chemical parameters of treated pork se'i were shown in Table 1.

Table 1. The average value of cholesterol in pork se'i treated with wistar

Treatment Group	Sample Code	Before Intervention	After Intervention	GAP	Average value and Standard Deviation	Sig.
Negative Control Group	K-1	50	20	-30	12,2 ± 39,72027 mg/dl	0.468
	K-2	49	60	11		
	K-3	44	91	47		
	K-4	53	110	57		
	K-5	51	27	-24		
Positive Control Group	K+1	58	12	-46	-29,8 ± 15,00667 mg/dl	0.359
	K+2	63	19	-44		
	K+3	60	48	-12		
	K+4	55	36	-19		
	K+5	61	33	-28		
Dose 0.89 gr pork se'i	PI1	55	30	-25	-2,6 ± 27,73626 mg/dl	0.767
	PI2	49	45	-4		
	PI3	36	51	15		
	PI4	54	21	-33		
	PI5	45	79	34		
Dose 1,78 gr pork se'i	PII1	47	32	-15	-6,4 ± 16,47119 mg/dl	0.553
	PII2	43	35	-8		
	PII3	56	32	-24		
	PII4	39	59	-20		
	PII5	41	36	-4		
Dose 3,56 gr pork se'i	PIII1	48	75	27	-20,6 ± 31,05318 mg/dl	0.482
	PIII2	45	38	-7		

	PIII3	63	12	-51		
	PIII4	55	21	-34		
	PIII5	59	21	-48		

2. Normality Test

The results of the descriptive analysis of changes in LDL values after and before the intervention showed that the significance of rats with positive control treatment induced using a high-fat diet was 0.468 > 0.05; negative control 0.359 > 0.05; the first treatment or P1 by giving 0.89 grams of pig sei every day of 0.767 > 0.05; the second treatment or P2 by giving 1.78 grams of pig sei every day of 0.553 > 0.05; and the third treatment or P3 by giving 3.56 grams of pig sei every day of 0.482 > 0.05. From the results of the significance analysis according to the Shapiro-Wilk test it was found that each variable was normally distributed.

3. Homogeneity Test

Homogeneity test using Levene's test. From this test obtained a significance value of 0.107, which means that the significance value >0.05. From the results of this test, it means that the research data comes from the same variant or is referred to as homogeneous data.

4. One Way ANOVA Test

The results of the analysis showed (1) the average difference in LDL cholesterol after and before the intervention in the positive control group induced by a high-fat diet was -29.8 mg/dl; (2) The mean difference between LDL cholesterol before and after the intervention in the negative control group that was not intervened with anything was 12.2 mg/dl; (3) The average difference in LDL cholesterol after and before the intervention in treatment group 1 (P1) which was intervened with 0.89 grams of industrial pig se'i every day was -2.6 mg/dl; (4) The average difference in LDL cholesterol after and before the intervention in the treatment group 2 (P2) which was intervened with 1.78 grams of industrial pig se'i every day was -6.4 mg/dl; (5) The average difference in LDL cholesterol after and before the intervention in the treatment group 3 (P3) which was intervened with 3.56 grams of industrial pig se'i every day was -20.6 mg/dl.

Descriptively, it can be concluded that the average difference in LDL cholesterol levels after and before the intervention in all treatment groups was -9.44 mg/dl with the difference in average LDL cholesterol levels after and before the intervention being the highest experienced by the negative control group of 12. 2mg/dl. There was no significant

difference in LDL cholesterol levels before and after the intervention between the positive control group and the negative control group.

The anomaly found in this study was that the positive treatment group which was given a high-fat diet consisting of 80% corn oil, 15% egg yolks, and 5% lard actually experienced a decrease in LDL cholesterol levels. The diet formula adapts the high-fat diet composition made by Levin and Dunn-Meynell (Levin and Dunn-Meynell, 2002). The formula given to experimental animals consisted of 68% rat food in the form of pellets, 20% milk powder substituted for egg yolk, 6% corn oil, and 6% ghee (milk solid fat) substituted for lard. The diet given to experimental animals was heated using an oven at 65°C for 24 hours. The treatment was given for 10 weeks to induce obesity in experimental animals. In the research conducted, the high-fat diet given to rats was not heated for 24 hours, but only for 15 minutes after stirring to avoid coagulation which would complicate the sonde process in experimental animals.

There is strong scientific evidence that LDL and Apo-B-containing lipoproteins including VLDL and the rest, such as IDL and lipoprotein A, directly implicate the development of atherosclerotic and cardiovascular disease (Goldstein and Brown, 2015). Biochemical markers that reflect the total amount of atherogenic lipoproteins are suggested to be examined in full in determining the risk of heart disease. LDL is the amount of cholesterol carried by LDL particles. LDL-C is the standard used to measure LDL and heart and blood vessel disease caused by LDL. This makes the terminology LDL and LDL-C used to replace one another (Otvos et al., 2011). LDL-C is not a sensitive biochemical marker for determining the risk of heart and blood vessel disease events because LDL-C is a biochemical marker that only provides a rough estimate of the concentration of LDL particles (LDL-P). Previous studies have provided clear indications that the risk of atherosclerosis is more determined by the number of LDL particles (LDL-P) compared to the total amount of cholesterol in LDL particles (Sigurdsson, 2021b).

Research has shown that consumption of foods with a high fat content (more than 40% of daily dietary intake) is a major cause of metabolic syndrome, increased serum metabolite concentrations, increased blood pressure, and increased inflammatory biomarkers. Chronic administration of a high-fat diet will cause an increase in triglycerides and glycerol by 29% and 19% when compared to mice that are not given a diet with a high fat content (Michael J et al., 2019). Obesity in rats induced by a high-fat diet progressively develops into fatty liver or hepatic steatosis, hyperlipidemia, low-grade inflammation, and

hypertension as conditions that can lead to an increased risk of stroke in experimental animals (Balti et al., 2008).

Cholesterol is present in the body in the form of free cholesterol or bound to long chain fatty acids and forms cholesterol esters. Cholesterol esters are synthesized from Acetyl-Co A and will be excreted from the body through bile salts. Free cholesterol will be removed from the network by HDL and transferred to the liver to be converted into bile acids. Hypercholesterolemia is characterized by an increase in blood cholesterol above normal. The normal cholesterol level in Rattus novergicus rats is 10-54 mg/dl.

Research by Harini and Astirin (2009) states that giving lard in a ratio (9:1) to the daily diet of mice will increase the rat's blood cholesterol content by 10.7%, LDL content by 55.52%, and there is no significant decrease in HDL, namely by 2.17%. This condition appears due to increased accumulation of fat in the liver of mice which has an impact on increasing the amount of acetyl CoA in the liver which increases cholesterol production (Guyton, 1991). Pork fat contains high saturated fat in the form of palmitic acid and myristic acid which causes triglycerides that come out of the liver to contain high levels of saturated fatty acids, causing an increase in blood cholesterol. Consuming large amounts of saturated fat will cause an increase in the ratio of total cholesterol to HDL thereby increasing the risk of atherosclerosis (Baraas, 1993). Consumption of too much fat was caused hyperlipidemia by increasing apolipoprotein B cholesterol and LDL. An increase in apolipoprotein B is usually associated with a decrease in LDL receptors in the liver so that the LDL content in the blood circulation will increase and decrease the rate of excretion (Verd et al., 1999).

Although this study was never intended to examine the genetic relationship with LDL size, the results show that there is a strong relationship between environmental components involved in determining LDL particle size. Changes in LDL size to be small or large are influenced by changes in fat. Lipoprotein and fat concentrations are regulated by a combination of genetics, environment, and hormones.

CONCLUSION

The study was that there was no relationship between the consumption of industrial pork se'i and an increase in LDL cholesterol in experimental animals.

Ethical approval and consent to participate

All experiments on this research were performed in accordance with relevant guidelines and regulations. This research has already been

approved by the assessor of Biochemistry and Physiology Laboratory Faculty of Medicine, Universitas Airlangga. This research has been approved by the ethical committee of the Faculty of Public Health Universitas Airlangga. The Ethics Committee of the Faculty of Public Health Universitas Airlangga have declared that with consideration of Human Rights and welfare protection in medical research, this research entitled “Relationship between pork sei consumption as a risk factor of dialysis in experimental animal” is deserve to be executed and have fulfilled a requirement to conduct animal based research. The letter mentioning this declaration is Letter of Ethical Approval Faculty of Public Health Universitas Airlangga No: 185/EA/KEPK/2022.

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Author Contribution

Maria Magdalena Dwi Wahyuni contributed in initiating the research in Kupang also providing basic data of pork sei consumption in Kupang NTT as the trigger of this research. Martina Puspa Wangi contributed in writing the articles, managing technical needs in the field during the experiment being conducted, and researching related to the protocol used in examining Bcl2 and p53 wild expression using Immunohistochemistry method. Trias Mahmudiono contributed in providing the fee during the research and giving guidance related to the written article. Indra Yohanes Killing and Marni Marni contributed in submitting and proofreading the article before being submitted to the journal. Sintha Lisa Purimahua contributed in managing the ethical clearance before the research was conducted.

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