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Weight Gain and Blood Glucose Level in Granting Trans Fatty Acids (Study at *Sprague Dawley)*

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Abstract

Obesity was often cause by excessive food intake, lack of physical activities, genetic susceptibility, endocrine disorders, psychiatry disease. Pathogenesis DM type 2 was disturbance insulin or lack of insulin which result of genetic factor interaction, environment and life style changes. The life style changes were increasing the intake of fast foods, snack foods hydrogenated, the amount of consumption of fried foods, which turned out to contain trans fatty acids, causing obesity and diabetes mellitus type 2. How interaction with weight gain and level of blood glucose was unclearly. The result of study was analyzing effect of ALT against to weight gain and level of blood glucose. The study was experimental with randomized controlled group pretest posttest design, conducted at LPPT Unit IV UGM Yogyakarta. The sample was 30 male Sprague Dawley rat and aged 8 weeks, weight 200-300 grams and divided into 3 groups , were control group, group of Treatment I with ALT granting 5% and group of Treatment II with ALT granting 10% during 8 weeks, and weight and level of blood glucose checked. The parameter difference before and after the intervention was analyzed with Wilcoxon testing. ALT granting 5% and 10% during 8 weeks increase weight and blood glucose level significantly. In conclusion**, t**he high dose of ALT increases weight and blood glucose.

***Keywords*:** ALT; Weight; Blood Glucose.

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1. Introduction

The prevalence of obesity in adults and children is increasing, so it is a serious health problem in the 21st century [1]. Obesity increases the chances of a wide variety of non-communicable diseases, especially heart disease, Type 2 diabetes, cancer, hypertension and stroke [2,3]. Obesity is caused by a combination of excessive food intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by genes, endocrine disorders, medications or psychiatric illness. Obesity is the leading cause of preventable death. [1]. The World Health Organization (WHO) estimates that being overweight in the near future will replace the public health diseases such as malnutrition and infectious diseases. In 2013, obesity in the world amounted to 2.1 billion and Indonesia entered as a sequence of top ten (10) with number 40 million people, equivalent to the entire population of West Java. Data Riskesdes in 2013 reported that the prevalence of obesity in the population aged more than 15 years was 10.3% (males 13.9% and females 23.8%). Obesity in developed countries generally suffered by men, but in Indonesia are mostly women [4].

Obesity is a risk factor associated with the disease diabetes mellitus type 2. There is strong evidence that underweight baby at birth tend to have insulin resistance, the risk of type 2 diabetes and metabolic changes in adulthood [5], while obese babies when born tend to be obese in adulthood [6]. Hyperglycemia is an indicator for the diagnosis of diabetes mellitus (DM), with the characteristics of blood glucose level increased exceed the normal value and continuous [7,8]. The prevalence of diabetes in the world and in Indonesia tends to increase with economic growth, especially type 2 diabetes mellitus [9]. Basic pathogenesis of type 2 diabetes mellitus is a disturbance in the action of insulin and the relative lack of insulin [10] which is the result of a complex interaction of genetic factors, environmental and lifestyle changes (Anonymous, 2008). Although the genetic basis of type 2 diabetes has not been identified, there is strong evidence that obesity and physical inactivity were a major determinant of non-genetic disease risk factors that can be modified [11].

Lifestyle changes, such as changes in eating patterns, from traditional food into Western diet, one of which is the increased intake of fast food (fast food) which contain high calories and fat [12], arouse diseases that are closely related to lifestyle (" Life Style Related Disease ") [13]. Fast food was not only containing high calories and fat, but it also contains high levels of trans fatty acids (ALT). ALT that many common is elaidic acid and linolelaidat formed from partially hydrogenated vegetable oils and deep frying process. Transvaccenat acid was ALT ruminant which produced by intestinal bacteria that found in adipose tissue, milk, meat and also results of processed such as butter, cheese [14].

ALT is also generated from the hydrogenation process in the manufacture of margarine, shortening and heating during oil processing. Hydrogenated oils also for cooking french fries, fast food products such as pizza, chips, crackers, snacks, cookies, instant noodles and others. Likewise, chips, cereals, margarine and biscuits processed using hydrogenated oils, so food product becomes more crispy,, creamy, more savory [15] and become community favour because it has good taste, savory, easy to rancidity, practical, portability because it was a semi-solid [16]. The traditional food is processed by frying (deep frying) like crackers, is the highest contributor to the intake of ALT in Indonesia. ALT consumption in the United States reaches 2-3% of the total intake energy. US Dietary Guidelines Advisory Committee recommended that ALT consumption for each individual is

under 1% of the energy total. Data about ALT levels and consumption in Indonesia is still not widely available, and a list of food composition (DKBM) Indonesia has not included the content of ALT. The attempts to obtain the content of ALT in food is analyzing in laboratory foods frequently consumed by the public. Results of previous studies in Jakarta, showed that the average intake of ALT was 0.71% from total energy. The results of analysis Kusmiyati to street food were often consumed by people in Semarang, which conducted at the Laboratory of Integrated Bogor Agricultural University (IPB), shows the content of ALT ranges (from 0.09 to 22.14%) from the total energy , Recommendations from the American Heart Association (AHA) to the intake ALT is <1% of the total energy [17]. ALT is currently very interesting because the effect is worse for your health than saturated fatty acids (ALJ) that were previously regarded as a type of fat that adversely affect health [18] .

Interaction between trans fatty acids and an increase in body weight and blood glucose levels is still controversial. Observational study in 2009 states that a high consumption of ALT may increase levels of HbA1c [19], whose levels are dependent on the concentration of blood glucose [20], on the other hand there is a controversial study, which states that a high intake levels of ALT does not affect the blood glucose [21].

The purpose of this study to analyze the increase in body weight and blood glucose levels in the provision of high ALT at Sprague Dawley.

2. Materials and Method

This study is an experimental laboratory study with laboratory penelitianeksperimental randomized controlled group pretest posttest design. The study was conducted in the laboratory of Integrated Research and Testing Unit IV Gajah Mada University (UGM) in Yogyakarta. The study population was 8 weeks Sprague Dawley were obtained from Unit IV LPPT UGM with consideration that rats is animal modes for learning changes in body weight and blood glucose levels. The rats was fed with high ALT 5% and 10%. Calculation of sample size refers to the formula Federer and WHO guidelines on the use of experimental animals and for experimental study obtained 10 animals per group.

Inclusion criteria: 200- 300 gram weight, active and healthy, fasting blood glucose (FBG) started from below 110 mg / dl. Exclusion criteria: diarrhea, change in weight below than than 10% or more than 10% for adaptation, visible pain during the treatment process. This study used 10 rats per group consists of: a control group (K1), the treatment I (K2): given ALT 5%, treatment II (K3) given ALT 10%. 8-weeks treatment based research Dorfman: an interruption metabolism of nutrients on the liver, adipose tissue and skeletal muscle. Rats are placed in individual cages, exposed to 12-hour light dark cycle, with sufficient ventilation, temperature 28-32^{0}C, daily cage cleaning.

Data are presented descriptively, the normality test data by Shapiro Wilks, then with the Wilcoxon test to analyze differences in pre and post treatment. Finding the homogeneity of the study subjects was randomized and analyzing level of blood glucose. BB rats were weighed every week. Blood specimens were taken from retroorbitalis plexus. Data are presented descriptively, the normality test data by Shapiro Wilks, then the

Wilcoxon test to analyze differences in pre and post treatment. To conduct the study subjects performed randomized and homogeneity analyze differences in GDP by Kruskal Wallis test, differ significantly, but still within normal limits.

3. Result

3.1 The rats weight

The changes of weight 0-8 weeks, displayed in Figure I

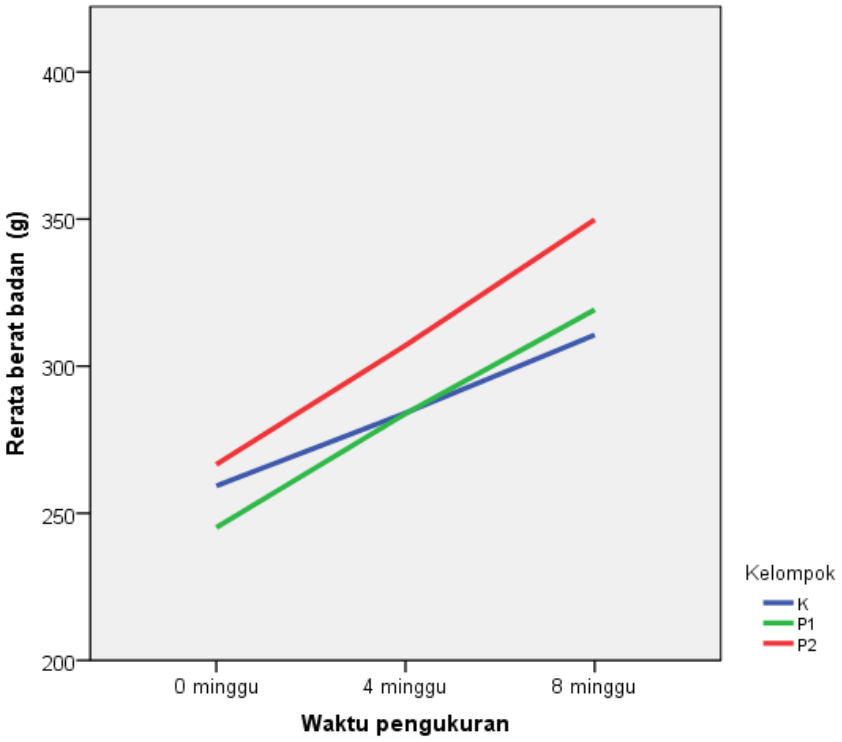

Figure 1: The changes of rats weight from 0 week-8 weeks

Table 1 shows that There are trend enhancement weight at Control Group (K1), Treatment I Group (K2) and Treatment II Group (K3), The average weight, the enhancement weight at 4-8 weeks in K1, K2 and K3.

Statistical analysis test showed weight rats at weeks 0 in group K1, K2, and K3 was not significant ($p > 0.05$). At 0-4 weeks rats in K1 and K2 groups were not differ significantly ($p > 0.05$), K1 and K3 were significant difference ($p < 0.05$)), K2 and K3 were significant difference ($p < 0.05$). In the 0-8-week inter-group K1, K2 and K3 were significant difference ($p < 0.05$), K1 and K2 wotere n differ significantly ($p > 0.05$), K1 and K3 were significant difference ($p > 0.05$), K2 and K3 were significant difference ($p < 0.05$). At 4-8 weeks in group K1, K2, K3 were significant difference ($p < 0.05$), K1 and K2 were not differ significantly ($p > 0.05$), K1 and K3 were significant difference ($p < 0.05$), K2 and K3 were not differ significantly ($p > 0.05$)

Tabel 1. The average weight (g), Δ enhancement weight and percentage changing weight at rats in K1(n=10), K2 (n=10) and K3 (n=10).

Time	Group			p
	K_1	K_2	K_3	
	The Average±SB; (g)	The Average ±SB; (g)	The Average ±SB; (g)	
0 week	259,3±21,11;	245,2±19,57;	266,6±17,84;	0,06§
4 weeks	284,1±25,58;	283,9±25,35;	307,1±10,88;	0,03¥
8 week	310,6±39,52;	319,2±23,94;	349,8±20,13;	0,01§
Δ0 s/d 4 weeks	24,8±8,39; p=0,00	38,7±16,73; p=0,01	40,5±12,78; p=0,02	0,02§
% the enhancement at 0-4 week	9,5	15,9	15,5	0,02§
Δ 0 s/d 8 weeks	51,3±24,55; p=0,00	74,0±18,97; p=0,00	83,2±23,40; p=0,00	0,01§
% the enhancement 0-8 weeks	19,6	30,5	31,6	0,01§
Δ 4s/d 8 weeks	26,5±13,94 p=0,00	35,3± 1,41 p=0,02	42,7± 9,25 p=0,00	0,00#
% the enhancement 4-8 weeks	9,32	12,43	13,9	0,00#

§ *One Way* ANOVA testing

¥ Kruskall-Wallis testing

Wilcoxon testing

SB=Standard deviation; Min=minimum; Maks=Maksimum

Δweight at 4 weeks: weight weeks 4- week 0

Δ weight at 8 weeks: weight weeks 8 week 0

Δ weight at 4-8 weeks: weight weeks 8- week 4

%the enhancement of weight at 4 weeks: (ΔBB 4 weeks/weight 0 week) X 100%

% the enhancement of weight at 8weeks: (ΔBB 8 weeks/BB 0 week) X 100%

% The enhancement weight 4-8 weeks: (ΔBB4-8 weeks/BB4 weeks) X 100%

3.2 The test of spearman correlation

Table 2 showed that K2 there was no significant correlation between higher intake ALT with rats weight and Δ rats weight. At K3 there was no significant positive correlation between higher intake ALT and weight and Δ weight. It means that more higher intake ALT will improve weight and delta of weight enhancement .

Table 2: The correlations between high intake ALT with weight and Δ enhancement weight until 8-weeks in K2 and K3 group

Variables		ALT		Interpretation
		rho	Ninai-p	
Weight	K2	0,297	0,405	There are not significant correlation
	K3	0,806	0,005	There are significant and positive correlation
Δ Weight	K2	0,491	0,050	There are not significant correlation
	K3	0,806	0,005	There are significant and positive correlation

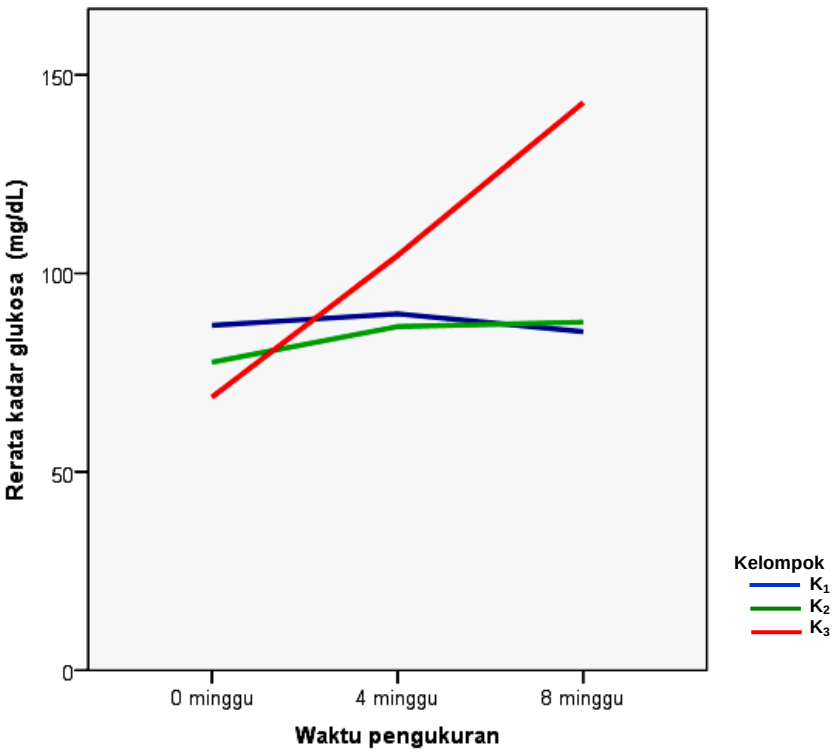

Figure 2: The changes level of blood glucose in K1, K2 and K3 from 0 weeks to 8 weeks shown

3.3 Levels of blood glucose

Changes in levels of blood glucose in K1, K3 K2 from beginning until 8 week shown in Figure 2.

In Figure 2 looks at the K1 level of blood glucose from 0 week to the 8th rise and fell back in 8 weeks. At K2 from beginning to 4 week there was a slight increase in levels of blood glucose and until 8 weeks there are still an increase, but the increase is not as bigger as the 0 week to 4 weeks. At K3, there are large increase levels of blood glucose since the 0 week to 8 weeks. .

The mean levels of GD at beginning, at 4 week and 8 weeks, , the average changing levels of blood glucose in K1, K2 and K3 shown in Table 3.

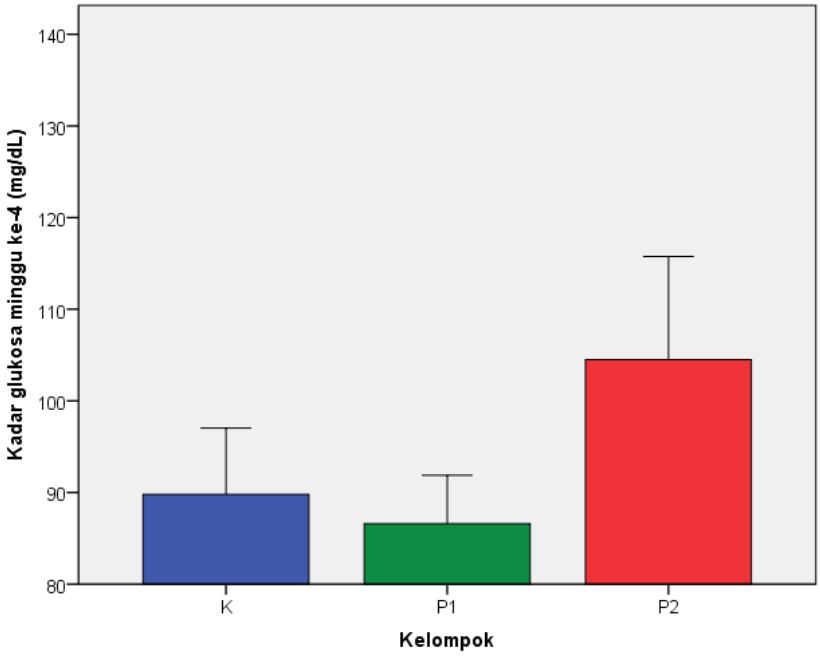

Figure 3: The level of blood glucose at in 4 weeks *Sprague Dawley at Group K1 (n=10), K2(n=10) and K3 (n=10). Value of p at table obtained from Mann-Whitney test*

Statistical analysis test showed weight of 0 week rats at group K1, K2, and K3 was not different significantly (p> 0.05). At 0-4 weeks rats in K1 and K2 groups were differ significantly (p<0,05), K1 and K3 were significant difference (p <0.05), K2 and K3 were significant difference (p <0.05). In the 0-8-week inter-group K1, K2 and K3 were significant difference (p <0.05), K1 and K2 were differ significantly (p> 0.05), K1 and K3 were significant difference (p> 0.05), K2 and K3 were significant difference (p <0.05). At 4-8 weeks in group K1, K2, K3 were not significant difference,K1 and K2 were not differ significantly (p> 0.05), K1 and K3 were significant difference (p <0.05), K2 and K3 were not differ significantly (p> 0.05).

The comparison average level of blood glucose after 4 weeks at 3 groups displayed at figure 3.

Table 3: Average levels blood glucose and changes blood glucose rats at K1 (n = 10), K2 (n = 10) and K3 (n = 10) were assessed at 4 and 8 weeks

Time	Groups			p
	K_1	K_2	K_3	
	The Average ±SB; (mg/dl)	The Average ±SB; (mg/dl)	The Average ±SB; (mg/dl)	
0 week	86,9±7,05;	77,6±10,65;	68,8±10,80;	0,001§
4 weeks	89,8±7,22;	86,6±5,28;	104,5±11,25;	0,001¥
8 weeks	85,3±3,30;	87,7±4,22;	143,0±8,17;	<0,001¥
Δ 0 s/d 4 week	2,9±7,58; p=0,25	9,0±6,13; p=0,26	35,7±12,18; p=0,00	<0,001¥
% the enhancement at 0-4 weeks	3,37	11,60	51,89	<0,001¥
Δ 0 s/d 8 week	-1,6±7,49; p=0,51	10,1±7,96; p=0,35	74,2±11,62; p=0,00	<0,001¥
% the enhancement at 0-8 weeks	1,84	13,02	107,85	<0,001
Δ 4-8 week	-4,5±3,92 p=0,08	1,1±1,06 p=0,60	38,5±3,08 p=0,00	0,000
% the enhancement at 4-8 weeks	5,01	1,27	36,84	0,000

§ *One Way* ANOVA test

¥ Kruskall-Wallis test

SB=Standar deviation; Min=minimum; Maks=Maksimum

Δ level of blood glucose at 4 weeks: level of blood glucose 0-4 weeks

Δ level of blood glucose at 8 weeks: level of blood glucose 0-8 weeks

Δ level of blood glucose at 4 weeks: level of blood glucose 4-8 weeks

At Figure 3 after 4 weeks, levels of blood glucose at K1 is not different significantly with K3 (p>0,05). Level of Blood glucose after 4 weeks, K3 is higher and significantly than K2 and K1 groups (p<0,05).

The comparison average level of blood glucose after 8 weeks at 3 groups displayed at Figure 4.

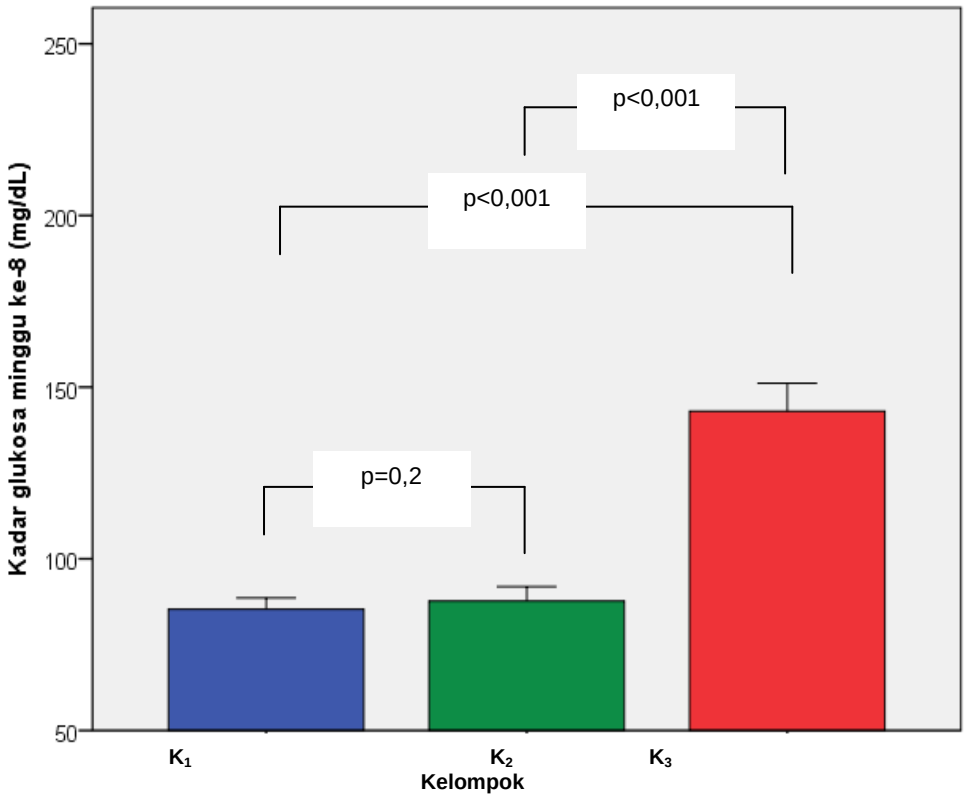

Figure 4: The level of blood glucose at in 8 weeks *Sprague Dawley at Group K1 (n=10), K2(n=10) and K3 (n=10). Value of p at table obtained from Mann-Whitney test*

At Figure 4 in 8 weeks, levels of blood glucose at K1 is not different significantly with K2 (p>0,05). Level of Blood glucose after 8 weeks, K3 is higher and significantly than K1 and K2 groups (p<0,05).

4. Discussion

4.1 The higher ALT intake and weight

At the beginning of treatment, weight of rats between 200- 300 g, and every week, rats are weighed using electric scales with the size of the gram, after 4-weeks the highest percentage increase in group 10% intake ALT followed by the standard intake group, and the lowest increase occurred in the group 5% intake ALT. In the 8-week, the largest percentage weight increase occurred in the group K3, although intake in this group is smaller than the intake on the standard group, increased weight at 5% intake ALT group higher than the standard intake group.

The weight growth is strongly influenced by the nutritional content of food, energy content, the amount of intake, metabolic activity, and physical activity of rats. The intake is supplied to provide maintenance energy requirements. Increased weight closely related to dietary habit diet, in this study is the high intake of ALT, in addition to other factors such as lack of physical activity. The energy content in the feed given to the three groups was of (4057) Kcal%, intake isocalorific in the form of formulas, in addition to containing the ALT also contain ALJ with a ratio of the number ALJ is 20 (in the standard intake group): 10 (in the 5% ALT intake group): 1 feed group ALT 10%. There is a significant positive correlation between higher intake ALT 10% with rats weight, also looked for a correlation between the intake of high intake have positive and significant correlation ALT 10% with a delta weight enhancement. It means that more high intake ALT will improve weight. In the group of standard intake, the intake contains ALJ, an increase in weight, and significantly different with 10% ALT group which containing ALJ in small quantities, weight significantly increase compared to the standard group. This shows that there is a relationship between increased weight and 10% ALT intake. ALT high intake of beta oxidation inhibit process, resulting in the accumulation of long-chain fatty acid CoA (LC-CoA) and inhibit the activity of uncoupling protein-2 (UCP-2), so that the energy release is inhibited, an increase in fat deposits, which resulted increase weight. 4-week treatment show increase weight in standard intake group and groups of intake ALT 5% and groups of intake ALT 10%. The highest increase occurred in the group with ALT intake of 10%, followed by the standard intake group, the next group with ALT intake of 5%. And it also occur in 8-week treatment, weight increased is greater in the group of 10% ALT intake compared than standard intake group and the group with ALT 5% intake. The Standard intake group occur a greater rise than the 5% intake ALT group . Increased weight at standard intake group caused by the content ALJ found in lard, which is the dominant containing stearic acid and palmitic acid. Palmitic and stearic fatty acid were a fatty acid that tends to easily deposited as subcutaneous fat [22]. There are significant differences between the groups ALT intake of 5% and 10%, thus it can be said that the increase weight at ALT group 5% and 10% due to high intake of ALT.

This study is in accordance with previous studies [23] which uses experimental animals apes, where the high provision ALT decrease insulin sensitivity associated with increased weight, abdominal obesity and decreased efficiency of insulin signal transduction at the receptor level postbinding. Other studies in rats [24-26] states that administration of high diet ALT causes insulin resistance, especially fat mass accumulation of triglycerides in the liver as a result decrease lipid oxidation and increased synthesis of fatty acids , which ultimately can lead to an increase in weight and obesity.Signaling ALJ and ALT in connection with proinflammatory effects which resulted in the development of insulin resistance is still pathophysiology unclearly. Some publications stated ALT signaling pathway and the ALJ have the same line, since the ALT has a structure similar with ALJ [27]. Statistical analysis showed that the standard intake group, 5% of intake ALT, ALT 10%, with the provision of 0-4 weeks, 4- 8-week, 0 wekk-8-weeks were significant difference ($p < 0.05$), indicating that longer and higher intake ALT effect to the increase of weight.

4.2 The higher ALT intake and blood glucose level

Blood Glucose levels in this study is one of the parameters indicate the presence of beta cell necrosis pankreas. The result of the study shows that the levels of the earlier Blood Glucose significantly different between groups, but still within normal levels (50-135 mg / dl). Blood Glucose levels at rats in accordance with previous literature [28] was 50- 135 mg / dl. Levels of blood glucose serum between groups at weeks 0-4, 0- 8 weeks, 4-8 weeks were significantly different. Standard intake group at 0-4 weeks, 0-8 weeks, 4-8 weeks and did not differ significantly. ALT intake 5% group in 0-4 weeks, 0-8 weeks, 4-8 weeks and did not differ significantly. ALT intake 10% group in 0-4 weeks, 0-8 weeks, 4-8 weeks were significantly different, the biggest increase occurred in the intake 10 % group at 8 weeks of treatment, the results of this study indicate that a more and higher ALT intake effect on elevated blood glucose levels. This is consistent with previous research [24] in experimental animals, states that the administration of a diet containing 10% ALT, induce insulin resistance through unclear way, trigger metabolic syndrome, weight gain, and cause accumulation fat mass, especially triglycerides in the liver. Previous research [29] states proinflammatory effects of ALT increase insulin resistance, endothelial cell function failure, increased lipid oxidation. Other studies demonstrate that administration of 20% ALT kkal causes increased insulin resistance in patients with type 2 diabetes mellitus, but it didn`t increase insulin resistance in thinner and healthy people [30], [31]. This is in contrast to other studies (Yamada et al., 2009) which states that the ALT diet was not associated with blood glucose level enhancement and body mass index, but it associated significantly with waist circumference, triacylglycerol levels, and hemoglobin glikasid. Hyperglycemia effect at pancreatic will reduce expression gene of duodenal homeobox-1 was a transcriptional regulator of insulin gene that causes disturbances in the final stages of insulin exocytosis [32].

Provision high ALT continuously is one of the factors increasing the formation of free radicals ROS / RNS, and cause oxidative stress and trigger lipid peroxidation so that the production NO increase, which causes necrosis of pancreatic beta cells, and the impact on the occurrence of hyperglycemia.

5. Conclusion

Feeding high intake from total energy 8 weight at Sprague Dawley increase weight and significantly levels of blood glucose.

6. Suggestion

The result of this study suggest than the further study discuss about the levels of GLUT-4, insulin resistance, lipid profile, especially triglyceride levels that has not been done in this study, which may provide a more comprehensive explanation.

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