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The Effect of Monosodium Glutamate on the Lee Index in Mice (Mus Musculus)

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Abstract

Obesity is the buildup of abnormal or excessive fat that can be detrimental to health. High flavor-flavoring consumption or monosodium glutamate (MSG) is one of the factors contributing to the increase in obesity of the Indonesian population. This study aims to analyze the effect of MSG on giving to Lee's index in mice. The Lee index is one of the indicators in determining mice obesity. The type of research was pure laboratory experimental research, with the design Post Test Only Control Group. The mice were divided into four random clusters. Each cluster contained six mice. The control cluster was given standard feed + drink, cluster I was given standard feed + drink + MSG 10.4 mg / 20gbb, cluster II was given standard feed + drink + MSG 20.8 mg / 20gbb and cluster III was given standard feed + drink + MSG 26 mg / 20gbb for 28 days. Day 29 was the weight lift and the length to calculate the lee index. Data normality analyze used Spahiro Wilk and the homogeneity test used Levene's test. The results of the variance homogeneity test showed that the value of p = 0.374, so that the lee index value of each cluster did not have a significant difference (p> 0.05). Then continued with the One Way Anova test, the value of p = 0.246 (meaningful if p <0.05). It could be concluded that the provision of MSG 4 grams, 8 grams, and 10 grams did not have a significant effect on the lee index value in mice between the control cluster and the treatment cluster.

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INTRODUCTION

According to The World Health Organization (WHO), obesity is defined as abnormal or excessive buildup of fat that can harm health (who, 2015). Obesity is a condition of imbalance between body height and weight resulting from excess body fat tissues, around the body organs and sometimes infiltrating into the body's organ (Listiyana & Mardiana, 2013). Obesity is a condition of someone who has a weight figure above the ideal weight figure (Schienkiewitz et al., 2017). Someone with a Body Mass Index (BMI) of 30 kg / m ^ 2 or more is generally considered obese. If the BMI is 25-29.9 kg / m ^ 2 it is called pre obesity. For Asian people, BMI is above 25 kg / m ^ 2 (Dhara & Chatterjee, 2015).

Baseline Health Research (Riskesdas) 2018 states that the prevalence of obesity in adults over 18 years has steadily increased from year to year since 2007. Based on the results of the 2018 Riskesdas National Institute Of Health Research and Development of the ministry of health, it shows the prevalence of obesity has increased since the three Riskesdas periods in 2007 %, (2013) 14.8% and (2018) 21.8%. The indicator of obesity in adults is the body mass index (BMI) above 27.0. Riskesdas states that the obesity rate in adolescent girls aged 13-15 years is 16.0%, aged 16-18 years is 13.5%. Meanwhile, obesity under the age of 5 was 8.4% in men and 7.7% in women. And the prevalence of central obesity at age ≥15 years is 18.8% (2007), 26.6% (2013), and 31% (2018) (Riskesdas, 2018).

The increase in obesity in the Indonesian population is due to an increase in unhealthy living patterns by Indonesians. Increased consumption of fast food (junk food), low physical activity, genetic factors, psychological factors, economic status, diet programs, age and gender are factors that contribute to a shift in energy balance and lead to the incidence of obesity (the kurdanti et al., 2015). Nutrition and health behaviors are important in the public health sphere. In principle, someone has healthy eating behavior if the various menus they consume provide balanced nutrition. Communities today have the tendency to eat out of doors in prestigious places with a menu choice that does not meet balanced nutrition. The preference for fast food such as burgers, pizza and fried chicken and other foods does not guarantee its nutritional needs (Dewantari, 2013). A large quantity of fast food (instant) is being exchanged, both in liquid and solid form (Kristianingrum, 2012). People will definitely select delicious and tasty foods that are practical in making them. The food is rich in additives. One of the additives is a dish better known as vetsin (Monosodium Glutamate) (Widyalita, 2014).

MSG is a food additive consisting of sodium glutamate salts which functions to enhance the taste of food. MSG is used in packaged foods without a label on them, so it can cause people to consume high concentrations of MSG because it is not included in packaged foods (ismail, 2012). Monosodium glutamate (MSG) has long been known and used in Indonesia as a flavoring agent for cooking, and has been able to strengthen and enhance the flavor and aroma of the staple foods cooked, so that the results of these foods taste more delicious and savory with a more attractive aroma. Monosodium glutamate is a sodium/ sodium from glutamic acid. Glutamic acid is a non-essential amino acid which is an important component of protein that the body needs (Tobi, 2019).

The results of the Riskesdas (2013) research indicates that nearly four out of five Indonesians consume more than one smoke a day with a 77.3% percentage. Kazmi et al (2017) explains that MSG can induce obesity that leads to diabetes. Bera (2017) also explains that MSG can influence humans to overeat, leading to obesity. According to scientists, MSG causes lesions in the brain and interferes with leptin processing. Leptin is a hormone that signals to the brain that you have had enough to eat, kills your appetite and burns calories. The problem with leptin signaling is called leptin resistance, and it is a factor in obesity. Meanwhile, the results of research by Thasia (2017) report that statistically giving monosodium glutamate for 28 days can cause weight loss and weight loss in the liver. Therefore, the researcher wanted to conduct

further research to determine the effect of MSG on the lee index in mice. Mice are one of the animals that are often used in research, especially in the health sector. This experimental animal was used because it had a high reproductive rate, and was easy to adapt to. At a low price, the structure of the mice was small so that it was easy to understand, and had characteristics similar to humans. Biological characteristics, behavior, and even human diseases may also be incorporated into the body of mice as experimental materials. The gene structure that is similar to humans also helps the results of research using mice as experimental animals more accurately (Putri, 2018).

METHODS

The design of this research was a true experiment, with a Posttest Only Control Group Design. Population and sample of this research used mice (Mus Muscullus), male gender, Balb-C line aged 2 months with a body weight of 20-40 grams obtained from the Laboratory of Animal Care, Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Semarang. The sampling technique of this research used was random (random sampling). The number of research samples for each cluster was determined using the formula Federer (1996) $(n-1)(k-1) \geq 15$, In this study 4 experimental clusters were determined with 3 treatment clusters and 1 control cluster. With the results of 6 mice for 4 cluster and 1 spare mice provided to anticipate the loss of the experimental unit in each cluster to 7 animals per cluster using the formula $N = n / (1-f)$. So the total number of synthesized samples of mice was 28.

All test animals were kept in a special cage with a density of 7 animals per cage. The number of cages used is 4 pieces, each measuring 35x20x10 cm, made of plastic, the top was covered with an iron net, the bottom of the cage was given husks which are replaced every three days. The number of drinking bottles (330ml) in each cage is placed at the top of the ring so that the tip of the beverage enters the inside of the cage. All test animals were given standard feed and given daily drinking ad libitum during the acclimatization and treatment periods. The treatment period was carried out for 28 days with the control cluster, namely normal, only given standard feed + drink, cluster I was given standard feed + drink + MSG 4 grams / day, cluster II was given standard feed + drink + MSG 8 grams / day, and cluster III was given standard feed + drink + MSG 10 grams / day. The dose of MSG that was given was converted to the dose of mice with the conversion of mice was 0.0026 (Laurence & Bacharach, 1964), so that the results for treatment cluster I were 10.4 mg / 20gbb, treatment cluster II was 20.8 mg / 20gbb and cluster treatment III was 26 mg / 20gbb.

As MSG treatment is diluted into aquadest, it first becomes MSG solution with the preparation of solution of each group as follows:

-Treatment I 4 grams = 0.4 ml / 20gbb
– Treatment II 8 grams = 0.8 ml / 20gbb
– Treatment III 10 grams = 1 ml / 20gbb

Then the MSG solution was given using a 22G oral gavage swab. Oral sonde is attached to the roof of the mouth above the mice, then slowly it is inserted into the esophagus and the MSG solution is inserted (Stevani, 2016).

After 28 days of different treatment, the 29th day of the mice were weighed and measured body length to calculate the lee index. Lee's index is a measure of obesity in mice. Rats were declared obese if the Lee index value> 300. The weight of the rats was measured using a scale (in grams) and their body length was measured using a weiji roll (in cm). The results of measuring the body weight of mice then calculated the body mass index using the formula:

$$\text{Index Lee} = [\![(Body\ weight\ (g)^{1/3} / Naso - anal\ length\ (cm)]\!] \times 10^3$$

The naso-anal length is the length of the nose to the anus (lee et al., 2011). Then performed physical euthanasia, namely by inhalation of

chlorophomes in a closed container after the mice fainted, cervical dislocation was performed. Naso-anal length is the length of the body from nose to anus (Lee et al., 2011). Then performed physical euthanasia, namely by inhalation of chlorophomes in a closed container after the mice fainted, cervical dislocation was performed.

The data analysis technique in this study uses the One Way Anova test formula. The requirements that must be met before the test is carried out are the normality test first. Normality tests are done to test whether a variable is normal or not. Because the data sample is ≤50, it uses the Saphiro Wilk test, provided that if the p value is> 0.05, the data can be said to be normally distributed (Riwidikdo, 2012). Then the Homogeneity Test was carried out. The homogeneity test is used to test whether the data is homogeneous or not in a One Way Anova test. If homogeneity is met, the researcher can carry t the advanced data analysis stage. If the P value is> 0.05, the data can be said to be of the same or homogeneous variance (Dahlan, 2015). Followed by the One Way Anova test to test an experimental design with a design of more than 2, with a significant value (p <0.05). This statistical test was performed with the SPSS 21.0 program on a computer. This research was conducted at the Laboratory of Biology study program (department), faculty of mathematics, natural sciences, Semarang state university and has been approved by the Health Research Ethics Commission (KEPK) Semarang State University with number: 186 / KEPK / EC / 2020.

RESULTS AND DISCUSSION

During the study, there were two mice that died from the cluster I on the day 27th and from cluster II on the 20th day. It was estimated that hypersensitive treatment was responsible for intracargic death. Some mice were able to adapt to treatment, while those unable to adapt will die. In the cage of cluster III, it was also found spined on the back of several rodents in the field. According to this result of research, the number of beds was 26, because in this study using random sampling is then that 24 mice were used in the study. Table 1. Descriptive Statistics of Lee's Index Value.

Tabel 1. Descriptive Statistics of Lee's Index Value.

Cluster	N	Mean	Median	SD	Minimum	Maximum
I	6	326	321	10	319	344
II	6	325	324	8.1	317	336
III	6	337	341	16	317	360
Control	6	322	324	16	297	341

After 28 days of treatment, the 29th day of the experimental results were processed by computer using the SPSS 21 program. Based on the first obtained results, the highest value of the lee index was in the cluster 337 given MSG 10 grams/day, followed by the cluster I with 4 grams/day MSG, the second cluster with MSG 8 grams/day, and the lowest lee index was the control cluster.

Tabel 2. Lee Index Normality Variable test

Experiment Cluster	N	p – value	Conclusions
I	6	0.055	Normal
II	6	0.333	Normal
III	6	0.474	Normal
Control	6	0.692	Normal

Data processing starts with a normal data test using statistical tests on each treatment cluster I, II, III and control cluster. The data for the Lee index value after the normality test using the Saphiro Wilk test showed that the p value was> 0.05. From these

results, it could be concluded that the lee index value in mice is normally distributed. After testing the normality of the data, the test continued for the homogeneity of the variance. From the results of the analysis on the Levene Statistic showed the results of the p value = 0.374 in the Sig column, so it can be concluded that the Lee index value data in mice have similar or homogeneous variants. Furthermore, the One Way Anova test was carried out.

Table 3. the Difference in the Effect of the Four cluster on the Lee Index Value in Mice

Dependent Variable	Count F	p – value
Lee Index	1.496	0.246

Based on table 3, the results of testing one way anova F test = 1.496 with p value = 0.246. Because p value is 0.246 <α (0.05), it was concluded that no significant difference between the effect of the MSG four experimental cluster given to the Lee value index, so the provision of MSG for 28 days has no effect on the Lee Index value in male mice lines. Balb-C.

Although all mice after measuring body weight and body length to calculate the lee index formula showed a value of> 300, which means obesity, but in the control group only 1 head had a lee index value of <300. In accounting for the index of Lee, it requires the value of weight and length of naso-anal are needed in mice. From figure 1 showed the average weight was comparable between groups, the lowest weight is reflected in control groups, followed by groups I, III and the highest average body weight in group II. In figure 2, it can be seen that the average body length of mice between groups is not much different. The shortest average body length is in cluster III, then followed by the control cluster, cluster I and the average body length of the longest mice is in cluster II. Obese mice had a larger body size and relatively similar length to the general mice (Muslikhah, 2014).

Chart 1 weight of mice day 29th

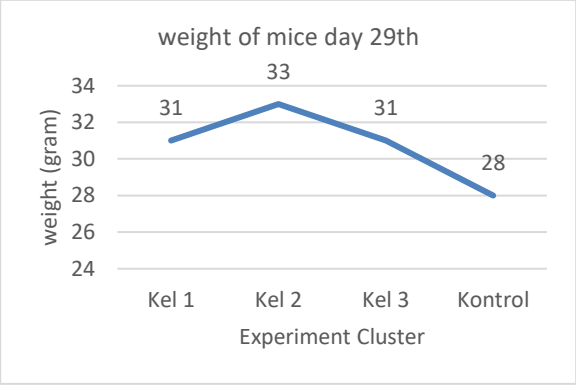


Chart 2 Leght of mice day 29th

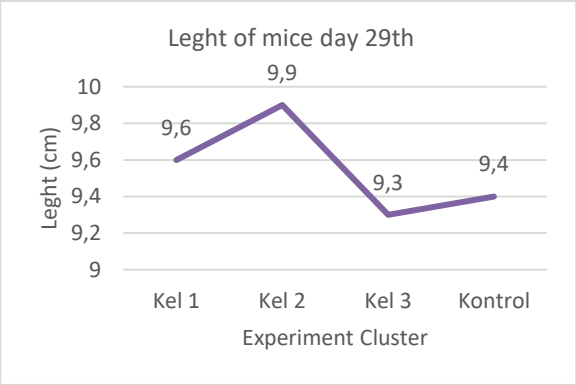
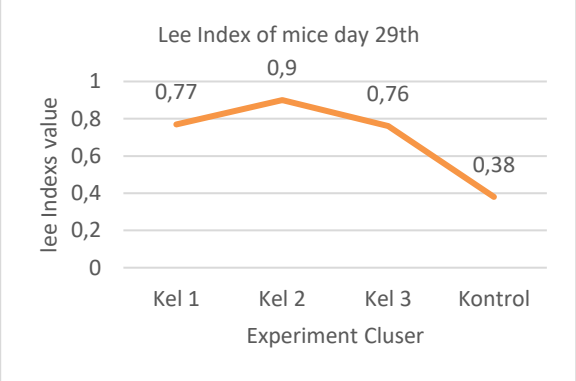


Chart 3 Lee Index of mice day 29th



Obesity is often due to more calories consumed than burning, the lack of physical activity can cause the deceleration of metabolism in the body, so less movement and the less calories will burn and the calories will accumulate in the body (agustin & thyme, 2019). The occurrence of obesity in mice in almost all experimental groups was due to an imbalance between energy intake and energy expenditure. The production of nutrients through

food given to snacks has a high density of energy, causing energy to overenter. The calories that do not convert to energy and unused are stored that lead to excess weight to obesity. Apart from the quantity of food, the mice were housed in a size of 35x20x10 cm with a density of 6-7 for each cage. This, of course, will make physical activity in mice limited. The lack of physical activity in mice will slow down the metabolism in the body so that the calories in the body will be more. accumulated a lot. The occurrence of obesity in almost all experimental animals is caused by the hormone obesity, namely the hormone leptin. When the experimental animal is obese, leptin resistance will occur. This case is due to a defect in the leptin receptor in the brain that does not respond to high levels of blood leptin which comes from a lot of fat tissue. Therefore, the brain does not detect leptin as a signal to decrease appetite until a higher benchmark point is reached or more fat stores. According to Salam (2010), obese people are more responsive than normal weight people to external hunger cues, for example the taste and smell of food or the time to eat.This coincides with the research conducted by Wasilah (2016) that the measuring of the body weight of rats before and after the study had a significant increase not due to the effect of MSG. The occurrence of obesity which causes weight gain in an individual is influenced, among others, by nutritional factors. Nutrition is basically the nutrient or nutrient found in the feed that passes through the individual's body asa feed consumption (Mardiati & Sitasiwi, 2016). Another source also says that MSG given through oral feeding is not shown any increase in body weight and the amount of fat, both in male and female mice. Meanwhile, giving MSG by injection has increased body weight and the amount of fat under the skin (Kurtanty et al., 2019).

This also aligns with the research of Thu Hien et al., (2013) that there was no significant relationship between MSG consumption and obesity. Boonnate et al., (2015) in his research also showed that there was no difference between the treatment cluster given MSG and the control cluster having almost the same body weight and food intake, Hernández-Bautista et al., (2014) also proved that mice given MSG body length can be shorter than the mice in the control group, 14% increase in body weight in female rats and 12% increase in body weight in male mice, for the lee index value also increased in the treatment group compared to the control group. However, contrary to the research of Kurtanty (2019), it was explained that in the study respondents who consumed the highest MSG would be obese by 30% compared to those who did not consume MSG.

CONCLUSION

Based on the result of this research, the concluded was there is no effect of MSG on obesity indicators of the Lee index in mice with a p-value of 0.246 ($p < 0.05$). There was no difference between the control cluster and the treatment cluster. The results of the study showed that the addition of MSG within 28 days did not make the mice test animals obese.

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