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Testing of Hypocholesterolemic Effects of Ledok Added with Seaweed in Vivo

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Abstract
Ledok is a traditional food in Nusa Penida, in form of porridge made from corn, cassava, beans, green leaves vegetables and seasoning without rice. *Ledok* has the potentials to be developed as a functional food, because it contents sufficient nutrients and bioactive component which is dietary fiber. The obyectives of this research is to discover the hypocholesterolemic effect of *ledok* added with seaweed *in vivo*. The experiment is conducted by Completely Randomize Design. The are four treatments which are : PO (rat group without hypercholesterol + standard feed = negative control), P1 (rat group with hypercholesterol + standard feed = positive control), P2 (rat group with hypercholesterol + standard feed + ledok 15 %) and P3 (rat group with hypercholesterol + standard feed+ ledok 30 %). Every group consists of six rats. Analysis are taken before (pretest) and after (posttest) feed treatment on total cholesterol, HDL, LDL, triglyceride of rat blood. The feed treatment is given for 28 days. The research shows that there is a lowering of cholesterol total level, triglyceride and cholesterol LDL of rats blood. In the other hand there is raise in rats blood cholesterol HDL level take place by giving mix of standard feed and *ledok* added with seaweed. Treatment of *ledok* by 30 % can lower total cholesterol level, triglyceride and LDL in the following order : 43.09 %, 42.30 % and 37.80 % from pretest level. Meanwhile HDL level of rat blood has a raise of 172,31 % from pretest level.
Keywords: *ledok*, hypocholesterolemic, cholesterol, triglyceride
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1. Introduction

Changes in consumption patterns of people who tend to consume foods which are high in fat and carbohydrates, but low in food fiber, has resulted in an increasing prevalence of degenerative diseases such as hyperlipidemia. This condition increases the risk of cardiovascular disease. Therefore controlling blood cholesterol concentrations is very important. One of the efforts that can be done is through diet therapy. In the diet therapy of hyperlipidemic patients, dietary fiber plays a very important role because it can reduce lipid levels. Soluble fiber can help improve glycemic control through a mechanism to delay gastric emptying. This response will also contribute to the reduction of blood lipids (McIntos *et al*. 2001). Consumer needs for functional food are increasing, because functional food has properties that can prevent certain diseases, beyond their nutritional functions (Subroto 2008).

Ledok is a type of porridge, one of the traditional foods in Nusa Penida, Klungkung Regency, Bali. *Ledok* is made from corn, cassava, beans, vegetables and herbs, without using rice (Suter *et al*. 2007). *Ledok* contains nutrients as follows: water (71.92%), ash (0.98%), protein (3.15%), fat (4.71%), crude fiber (3.18%) and carbohydrates (16.05%) (Suter *et al*. 2007), while instant *Ledok* porridge is made by adding 50% purple sweet potato contains water (79.01%), ash (1.26%), protein (6.67 %), fat (4.59%), and carbohydrate (8.46%), and antioxidant capacity of 0.10% (GAEAC) (Suter, et al, 2013). Furthermore Suter *et al*. (2013) reported that instant *Ledok* contains soluble food fiber of 2.08%, insoluble food fiber of 19.34% and total dietary fiber of 21.42%.

Ledok, which is developed into a functional food in addition to its high nutritional content, also contains bioactive components such as dietary fiber which play a role in the body's physiological processes. Increased reserves of *Ledok* bioactive components have been carried out by Suter *et al*. (2013) by using purple sweet potato and by Yusa *et al*. (2017a) using beans. The nutrient content of instant *Ledok* (*Ledok* porridge) is 80.68% air, 0.91% ash, 7.32% protein, 1.81% fat and 9.29% carbohydrate (Suter *et al*. 2009), dietary fiber has the preventive nature of degenerative diseases such as diabetes, coronary heart disease and high blood pressure (Kusharto 2006 & Subroto, 2008). Soluble dietary fiber has a hypocholesterolemic properties that can lower cholesterol levels (Stark & Madar 1994). It is also reported that high-dietary fiber can reduce blood glucose and lipid levels in patients with type 2 diabetes mellitus (McIntos *et al*. 2001, Kim *et al*. 2008). Testing of the hypocholesterolemic effect of *Ledok* made with the addition of red beans has been carried out by Yusa *et al*. (2017a) and the results showed that giving 20% of the total mixture of standard feed and *Ledok* can reduce cholesterol, triglyceride and HDL serum blood levels in experimental rats. Dietary fiber can lower cholesterol levels through several mechanisms including: (1) dietary fiber inhibits the absorption of cholesterol, (2) dietary fiber lowers the availability of cholesterol so that the transfer to the blood flow is reduced, (3) dietary fiber can prevent the synthesis of cholesterol and (4) dietary fiber can increase bile excretion (Marsono 2004).

Seaweed is one of the types of foods that contain high fiber which ranges from 25 - 75%, useful for people with hyperlipidemia, namely abnormal cholesterol levels, where LDL concentration is higher and HDL is lower than normal (Lahaye 1991 and Murata *et al*. 1999) and sufferers of hypertriacylglycerolemia (Murata *et al*. 2002). According to MacArtain *et al*. (2007), species of *E. cottoni, Gelidium sp*. and *Sargasum sp.* has a fiber content of 64.43%, 53.05% and 56% respectively. Seaweed species that have been widely cultivated by local farmers are *Eucheuma cottonii, Eucheuma spinosum* and *Gracilaria sp.* (Herpandi *et al*. 2006). Because of the content of seaweed fiber, seaweed is very potential to be used as a building block material to be developed into functional food. According to Yusa *et al*. (2017b) the type of seaweed of *Gracilaria sp.* produces *Ledok* with the best characteristics, namely the level of preferences based on color, aroma, texture, taste and overall acceptance level is "somewhat like", fat content of 0.11%, protein of 2.54%, carbohydrate of 15.28%, antioxidant capacity of 66.24 ppm GAEAC, IC-50 155.32 mg / ml, soluble dietary fiber of 2.80% db, soluble fiber food of 15.68% db, total food fiber of 18.40% db, mineral Ca of 2,620.72 ppm and Mn of 17, 18 ppm. However, it was not known whether *Ledok* that was made with the addition of *Gracilaria sp.* has hypocholesterolemic properties.

On the basis of the description above, further research is needed to examine whether *Ledok* made with the addition of *Gracilaria* seaweed has a hypocholesterolemic effect, which can reduce blood serum total cholesterol levels. The purpose of this study was to determine the hypocholesterolemic effects of the *Ledok* diet made with the addition of *Gracilaria sp*. on the experimental rats.

2. Materials and Methods

2.1. Materials and Tools

The materials used to make *Ledok* are: corn, corn flour, cassava, long beans, bay leaves, basil leaves, spinach leaves, garlic, salt, chili, seaweed of *Gracilaria sp*. and lemon. The chemicals used for the analysis are: reagent kits (cholesterol, triglycerides, and HDL). Rat feed used refers to the standards set by the American Institute of Nutrition (AIN 1993) including corn starch, CMC, soybean oil, sucrose, casein (Sigma, US), a mixture of vitamins and mineral mixtures (ICN Biomedical, Inc. Aurora, Ohio, America).

The equipment used is *Ledok* cooking equipment such as gas stoves, knives, blenders (Philips), and pans. The equipment used for chemical analysis includes homogenizer, vortex, small centrifugation (Hettich EBA III). Rat cages and its equipment.

2.2. Implementation of Research

a. Experimental design

The type of research is laboratory experimental in Wistar rats using research design namely Control Group Post Test Design (Notoatmodjo 2002 in Maligan *et al*. 2011). The selection of research objects and administration of treatment used the Complete Random Design method. This experiment used four treatments as follows:

P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)

Each treatment consisted of six rats, so the total sample of all treatments was 24 Wistar rats.

b. Ways of making *Ledok*

The formulation and method of making *Ledok* in this study refers to the formulation and method of making *Ledok* reported by Yusa *et al*. (2017b) as follows. Dry *Gracilaria* seaweed is cleaned and sorted, then weighed as much as 100 g, washed with water four times, then soaked in 1% citric acid solution for 24 hours. The amount of citric acid solution used is one liter or the ratio of the weight of seaweed and citric acid solution is 1: 10. After soaking, seaweed is washed four times with clean water, then drained. Next, seaweed is cut into small pieces. Vegetables (spinach leaves, basil leaves and bay leaves) are washed with water. All *Ledok* materials are weighed according to the formulation (Table 1). Spices, namely chili, garlic, onion, lime skin and salt mixed are then blended.

The process of making *Ledok* is as follows: Water is heated to boiling, then corn is added and boiled until half cooked (15 minutes), then other ingredients are added except for spinach and basil leaves which are added lastly. Boiling continues until the mature *Ledok* becomes porridge (20 minutes).

Table 1. *Ledok* formulations (Yusa *et al*. 2017b)

Num.	Ingredients	Amount (g)
1	White corn	100.00
2	Cornflour	52.50
3	Cassava	90.00
4	Chili	3.00
5	Shallots	5.00
6	Garlic	1.50
7	Peel of kaffir lime	0.25
8	Spinach leaf	150
9	Beans	107.50
10	Basil leave	10.00
11	Bay leaf	2.00
12	Salt	3.00
13	Seaweed (*Gracilaria sp.*)	39.00
14	Water (ml)	1.71

c. The Making of standard feed and treatment feed

The making of standard feed refers to the manufacture of standard feed according to AIN 1993 (Reeves *et al*. 1993). The composition of ingredients for standard feed can be seen in Table 2. The standard feed is made as follows: All ingredients are mixed and then the mixture is made by adding 250 ml of water. The mixture is then ground with a meat grinder to get the mill in the form of a pellet. Pellets are dried in the oven at 70^{o} C until dry. *Ledok* powder was made according to the method reported by Yusa *et al*. (2017a), i.e. *Ledok* porridge is dried in an oven at 70^{o} C until dry. The dry *Ledok* is then blended and sieved with a 16 mesh size sieve to obtain *Ledok* powder. The *Ledok* treatment is made by adding 15% of *Ledok* powder to the standard feed (P2) and adding 30% of the *Ledok* powder to the standard feed (P3).

Table 2. Standard feed composition (Reeves *et al*. 1993)

Ingredients	Standard feed (g / kg)
Cornflour	620.69
Casein	140.00
Sucrose	100.00
Soybean oil	40.00
CMC	50.00
Mineral mix	35.00
Vitamin mix	10.00
L-cysteine	1.80
Choline bitartrate	2.50
Total	999.99

d. Bioassay

The rats used in this study were Wistar rats with a body weight ranging from 150-200 g, totaling 24 rats. Rats were placed in individual cages and adapted to the standard feed for four days. On day 5, rats were fed with hypercholesterol feed (containing 1% cholesterol and 0.01% propyl thio uracil) for two weeks, except the negative control group (P0) were not given hypercholesterol feed. During the administration of hypercholesterol feed, the rats were also given duck egg yolks in a rounded manner i.e. 0.25 ml on the first day to the third day, 0.5 ml on the fourth to sixth day, 0.75 ml on the seventh to ninth day and 1 ml on the 10th day to 14th day. After two weeks of administration of hypercholesterol feed, an analysis of total blood serum cholesterol was carried out to ensure that the rats had positive hypercholesterolemia. Besides that, it was also carried out an analysis of triglycerides, LDL and HDL as well as weighing the rats (pre-test). Rats were divided into three groups, each group consisting of six rats. Each group was given feed according to the treatment, namely P1, P2 and P3. The treatment test was carried out for four weeks (28 days). Analysis of lipid profiles (total cholesterol, LDL, HDL, and triglyceride levels) was carried out before the pretest feed was given and four weeks after the treatment (Post-test). Observation of feed consumption was done every day, while weighing rats was carried out every week. The bioassay procedure can be seen in Figure 1.

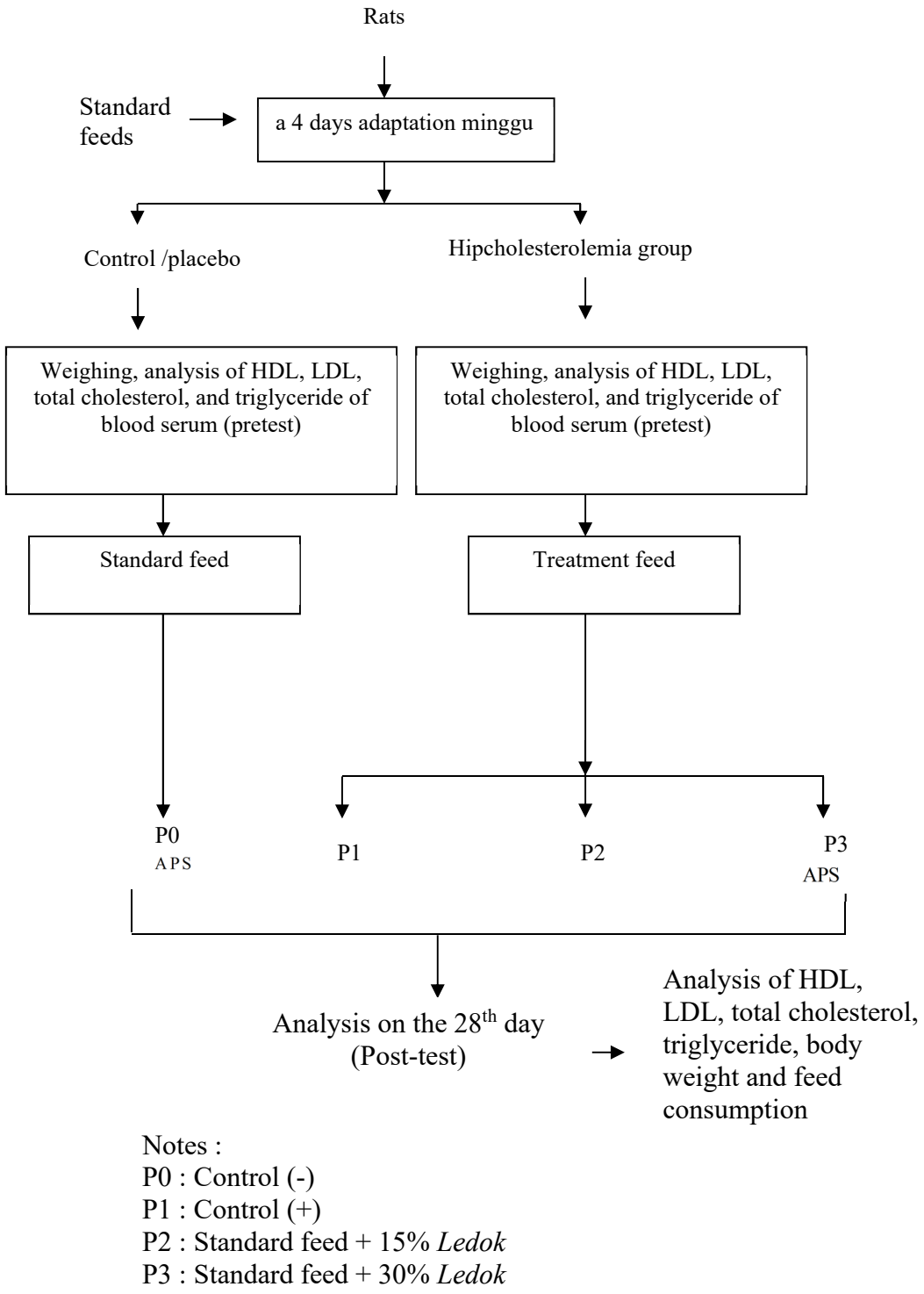

Figure 1. Bioassay procedure

e. Parameters Observed

Parameters observed/measured in experimental animals were rat body weight, amount of feed consumed and lipid profile. Lipid profile analysis includes total serum blood cholesterol with the CHOD-PAP method (Deeg *et al*. 1983 and Artiss *et al*, 1997), HDL with the CHOD-PAP method (Lopes-Virella *et al*. 1997), triglycerides with the GPO-PAP method (Fossati & Principe 1982), LDL was calculated by the [LDL-chol] equation = [total chol] - [HDL-chol] - [TG]/5 (Friedewald *et al*. 1972). Data from observations were analyzed statistically (Gomes & Gomes 1995).

3. Results and Discussion

3.1. Feed Weight Consumed

The average weight of feed consumed by the pretest (before given treatment feed) and post-test (after being given treatment feed) is presented in Table 3. Based on the data in Table 3, it can be seen that during the experiment there was an increase in the amount of feed consumed by rats which ranged from 85.70% to 100.70%. The Increasing amount of feed consumed by rats was greater in the group of rats given the treatment feed compared to the group of rats without treatment feed. This might be caused by the treatment of food added with *Ledok* which caused the aroma and / or taste of feed to be more attractive to rats so that it was consumed more.

Table 3. Average value of feed weight consumed by rats

Treatments*	Pretest (g)	Posttest (g)	Enhancement (g)	Percentage (%)
P0	8.67	16.10	7.43	85.70
P1	8.50	16.08	7.58	89.18
P2	8.50	17.06	8.56	10.70
P3	8.67	17.32	8.65	99.77

*P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)

3.2. Rats' Body Weight

The mean values of pretest and posttest rat weight are presented in Table 4. At the pretest of the hypercholesterolemic rat group, their body weight was significantly more (P <0.05) compared to the rat body weight of the rats without a hypercholesterol diet (negative control) (Table 4). This might be due to higher cholesterol-rich feed that causes higher body weight even though the weight of feed consumed is relatively the same. In the group of rats given additional cholesterol, the body weight of the rats was not significantly different (P> 0.05). In the post-treatment feeding process, i.e. mixed feed between standard feed and *Ledok* with a ratio according to treatment, caused differences in rat body weight.

Table 4. Average value of rat body weight

Treatments*	Pretest (g)	Posttest (g)	Enhancement (g)	Percentage (%)
P0	194.17±9.06b**	225.3±9.35b	31.13	16.03
P1	218.50±12.49a	250.2±13.04a	31.70	14.51
P2	221.67±12.24a	257.2±12.92a	35.53	16.03
P3	211.17±7.11a	245.3±7.84a	34.13	16.16

*P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)
**The same letter behind the average value in the same column showed no significant difference (P>0,05) based on DMRT.

In the hypercholesterol group of rats the body weight was significantly different (P <0.05), which was higher than the rat body weight of rats without hypercholesterol, but among the hypercholesterolemic rat group given standard feed and feed mixture of *Ledok*, the rat body weight was not significant (P> 0.05) i.e. ranged between 245.3 g - 257.2 g. There was an increase in rat body weight during the experiment which ranged from 14.51% to 16.16% to the pretest rat body weight due to an increase in the amount of feed consumed by rat during the experiment (Table 3).

3.3. Total Cholesterol Levels of Rats' Blood

The average value of total cholesterol levels in pretest and posttest rats' blood is presented in Table 5. At pretest, the administration of hypercholesterol diet showed significant differences (P <0.05), namely total blood cholesterol levels of rats in the group without being given a lower hypercholesterol diet compared to those given a hypercholesterol diet. Total cholesterol levels of rats given a hypercholesterol diet, among groups of rats given standard feed and groups of rats fed with *Ledok* mixed feed were not significantly different (P> 0.05) which ranged between 205.53 mg / dl and 206.74 mg / dl. Based on the data in Table 5 on the posttest, it can be seen that the total blood cholesterol level of rats in the rats given the treatment feed was significantly different (P <0.05), which was lower than the group of rats without given treatment feed (positive control). If it is compared between pretest and post-test, there was a decrease in total blood cholesterol levels of rats in the group of rats given standard feed

mixed with 15% and 30% *Ledok*, which were 32.39% and 43.09% respectively, whereas in the rat group without given treatment feed (positive control), there was a slight increase in total cholesterol levels of 2.74%.

Table 5. Average value of total cholesterol levels in rats' blood

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Enhancement/Reduction (mg/dl)	Percentage (%)
P0	74.13±2.40b**	75.49±2.88d	1.36	1.83
P1	206.74±4.49a	212.40±3.98a	5.66	2.74
P2	206.14±3.02a	139.37±6.40b	-66.77	-32.39
P3	205.53±3.81a	116.96±5.30c	-88.57	-43.09

*P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)
**The same letter behind the average value in the same column showed no significant difference (P>0,05) based on DMRT.

The decrease in total cholesterol levels of rat blood in the group of rats given *Ledok* may be due to the presence of dietary fiber in *Ledok*. Dietary fiber can reduce blood cholesterol levels (Rusilanti & Kusharto 2007). *Ledok* made with the addition of *Gracilaria sp*. seaweed containing a total dietary fiber of 18.40% which includes soluble dietary fiber of 2.80% and insoluble dietary fiber of 15.68% (Yusa, et al., 2017b). In foods containing high dietary fiber, there is an increase in excretion of fats, bile acids and cholesterol (Anderson *et al*. 1994). Dietary fiber can bind blood cholesterol and released through feces so that blood cholesterol decreases (Wisaniyasa 2017).

3.4. Rat Blood Triglyceride Levels

The average data of pretest and posttest rat blood triglyceride levels are presented in Table 6. Rat blood triglyceride levels in hypercholesterolemic group of rats were significantly different (P <0.05), which was higher than rat blood triglyceride levels in groups of rats without hypercholesterolemia. Blood triglyceride levels in hypercholesterolemic rats ranged from 125.90 mg / dl and 129.45 mg / dl. Based on the data in Table 6 on the post-test, it can be seen that the rat blood triglyceride levels in the rats given the treatment feed of the mixture of standard feed and 15% and 30% *Ledok* were significantly different (P <0.05) which was lower than the rats without treatment feed (positive control).

Table 6. Average rat blood triglyceride levels

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Enhancement/Reduction (mg/dl)	Percentage (%)
P0	67.60±1.98c**	68.80±1.60c	1.20	1.77
P1	129.45±2.51a	132.05±3.45a	2.60	2.01
P2	127.21±1.41ab	86.04±3.96b	- 41.17	- 32.36
P3	125.90±2.84b	72.65±5.30c	-53.25	- 42.30

*P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)
**The same letter behind the average value in the same column showed no significant difference (P>0,05) based on DMRT.

If it is compared between pretest and post-test, there was a decrease in rat blood triglyceride levels in the group of rats given standard feed mixed with 15% and 30% *Ledok* which were 32.36% and 42.30% respectively, whereas in the rat group without treatment feed (positive control) had a slight increase in triglyceride levels of 2.01%. This decrease in triglyceride levels may be due to the effect of dietary fiber on *Ledok*. Consumption of dietary fiber that meets the needs can increase fat excretion through feces (Brown *et al*. 1999). Dietary fiber can bind fat, so that lower fat absorption also means lowering blood triglyceride levels.

3.5. HDL Level of Rat Blood

The average HDL levels of pretest and post-test rat blood are presented in Table 7. The HDL levels of rat blood in hypercholesterolemic group of rats were significantly different (P <0.05) which was lower than the HDL level of rat blood in the rat group without hypercholesterolemia. HDL levels of rat blood in hypercholesterolemic rats ranged from 24.49 mg / dl and 25.51 mg / dl. Based on the data in Table 7 on the post-test, it can be seen that the HDL levels of rat blood in the group of rats given treatment feed with a mixture of standard feed and *Ledok* of 15% and 30% were significantly different (P <0.05) which was higher than the group of rats without treatment feed (positive control).

Table 7. Mean value of HDL levels of rat blood

Treatments*	Pretest (mg/dl).	Posttest (mg/dl).	Enhancement/Reduction (mg/dl)	Percentage (%)
P0	82.54±3.24a**	80.68±2.80a	- 1.86	- 2.25
P1	25.51±1.65b	24.27±1.24d	- 1.24	- 4.86
P2	25.17±0.96b	49.23±3.11c	24.06	95.59
P3	24.49±2.19b	66.69±2.67b	42.20	172.31

*P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)
**The same letter behind the average value in the same column showed no significant difference (P>0,05) based on DMRT.

If it is compared between pretest and post-test, there was an increase in HDL levels of rat blood in the group of rats given standard feed mixed with *Ledok* of 15% and 30%, which were 95.59% and 172.31% respectively, whereas in the rat group without treatment feed (positive control) decreased slightly in HDL levels by 4.86%. This increase in HDL levels may be related to a decrease in rat blood triglyceride levels.

3.6. LDL Level of Rat Blood

The mean LDL levels of pretest and post-test blood rats are presented in Table 8. At the pretest of LDL blood levels of rats in hypercholesterolemic group of rats were significantly different (P <0.05) which was higher than LDL blood levels of rats in the group of rats without hypercholesterolemia. LDL levels of rat blood in hypercholesterolemic rats ranged from 74.74 mg / dl and 76.47 mg / dl. Based on the data in Table 8 on the post-test, it can be seen that the LDL levels of rat blood in the rats given the treatment feed with a mixture of standard feed and *Ledok* of 15% and 30% were significantly different (P <0.05), which was lower than the rat group without given treatment feed (positive control). If it is compared between pretest and post-test, there was a decrease in LDL blood levels of rats in the group of rats given standard feed mixed with *Ledok* of 15% and 30%, which were 26.99% and 37.80% respectively, whereas in the rats group without treatment feed (positive control), there was an increase in LDL levels of 2.75%.

Table 8. Average value of LDL levels of rat blood

Treatments*	Pretest (mg/dl)	Posttest (mg/dl)	Enhancement/Reduction (mg/dl)	Percentage (%)
P0	23.65±2.12b**	24.94±2.26d	1.29	5.45
P1	76.47±1.43a	78.57±2.46a	2.10	2.75
P2	75.78±1.50a	55.33±2.00b	- 20.45	- 26.99
P3	74.74±1.69a	46.49±2.42c	- 28.25	- 37.80

*P0: The group of rats without hypercholesterolemia + standard feed (negative control)
P1: The group of hypercholesterolemic rats + standard feed (positive control)
P2: The group of hypercholesterolemic rats + standard feed + *Ledok* (15%)
P3: The group of hypercholesterolemic rats + standard feed + *Ledok* (30%)
*The same letter behind the average value in the same column showed no significant difference (P>0,05) based on DMRT.

Decreased blood LDL levels in the group of rats given the *Ledok* mixed diet may be due to the effects of dietary fiber found in *Ledok*. As discussed earlier, the feeding with *Ledok* can reduce total blood cholesterol levels in rats. Decreasing total cholesterol levels means there is also a decrease in blood LDL levels (Indra & Panunggal 2015).

4. Conclusions

This research can be summarized as follows:

1. The feeding with mixed feed namely standard feed and *Ledok* added with seaweed of *Gracilaria* sp. resulted in an increase in both the amount of feed consumed and the body weight of rats during the 28 day experiment.
2. Feeding with mixed feed namely standard feed and *Ledok* added with seaweed of Gracilaria sp. effect on total cholesterol, triglycerides, LDL and HDL levels, in which there was a decrease in the levels of total cholesterol, triglycerides and LDL in rat blood, while HDL levels in rat blood increased.
3. Feeding with mixed feed namely standard feed and 30% *Ledok* can reduce total cholesterol, triglyceride and LDL levels in a row by 43.09%, 42.30% and 37.80% from the pretest level, while the HDL level of rat blood increased by 172.31% from the pretest level.

From the research results, it can be suggested that *Ledok* which is made by adding seaweed Gracilaria sp. can be used as a diet for people who experience hyperlipidemia disorders.

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