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The tuber extract and flour of *Dioscorea alata* normalize the blood lipid profile of rabbits treated with high cholesterol diets

Nelis Imanningsih,¹ Deddy Muchtadi,² Nurheni S. Palupi,² Tutik Wresdiyati,³ Komari¹

¹ National Institute of Health Research and Development, Ministry of Health, Jakarta, Indonesia

² Department of Food Science and Technology, Bogor Agricultural Institute, Bogor, Indonesia

³ Department of Veterinary Science, Bogor Agricultural Institute, Bogor, Indonesia

Corresponding author: Nelis Imanningsih

E-mail: nelisimanningsih@gmail.com

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Abstrak

Latar belakang: Umbi *Dioscorea alata* (DA) memiliki potensi untuk mencegah kondisi hiperlipidemia karena mengandung senyawa bioaktif antosianin, serat pangan, dan diosgenin yang bermanfaat dalam menormalkan kadar lipida darah. Pada penelitian ini dipelajari pengaruh pemberian ekstrak air dan tepung umbi DA dalam menormalkan profil lipida darah kelinci yang diberi ransum tinggi kolesterol.

Metode: Ekstrak dan tepung umbi DA diberikan kepada kelinci percobaan selama 60 hari dengan disain penelitian rancangan acak lengkap. Perlakuan ransum sebagai berikut: 1) Ransum basal sebagai kontrol negatif (K0), 2) Ransum basal + kolesterol 0.5% sebagai kontrol positif (K1), 3) Ransum basal+kolesterol 0.5% + ekstrak DA 1.8 g/100 g (KE1), 4) Ransum basal+kolesterol 0.5%+ekstrak DA 3.6 g/100 g (KE2), 5) Ransum basal mengandung 15% tepung DA + kolesterol 0.5% (KT1) dan 6) Ransum basal mengandung 30% tepung DA + kolesterol 0.5% (KT2). Kadar total kolesterol, LDL, HDL kolesterol dan trigliserida pada serum dianalisa pada awal, hari ke 28, hari ke 56 dan pada akhir penelitian.

Hasil: Pemberian ransum tinggi kolesterol sebanyak 1% meningkatkan kadar kolesterol darah sebesar 16 kali dibandingkan kontrol. Pemberian 15% dan 30% tepung DA ransum tinggi kolesterol dapat mempertahankan profil lipida darah kelinci ke arah normal, khususnya pada substitusi 30% tepung DA. Akan tetapi, ekstrak air tepung DA tidak dapat mempertahankan kadar lipida normal kelinci yang diberi ransum tinggi kolesterol.

Kesimpulan: Tepung DA memiliki efek anti-hiperlipidemia. (*Health Science Indones 2014;1:23-9*)

Kata kunci: ekstrak, tepung, *Dioscorea alata*, hiperlipidemia

Abstract

Background: *Dioscorea alata* (DA) tuber has potential to prevent the condition of hyperlipidemia due to the bioactive compound, such as anthocyanins, diosgenin, and dietary fiber that beneficial in normalizing blood lipid profiles. In this research, the effect of water extract and flour of DA tuber administration was examined on rabbits treated with high cholesterol diets.

Methods: DA tuber extract and flour were administrated to the rabbits for 60 days using completely randomised design. The ration treatment are as follows: 1) Basal ration as negative control (K0), 2) Basal ration + 0.5% cholesterol, as positive control (K1), 3) Basal ration + 0.5% cholesterol + DA extract 1.8 g/100 g (KE1), 4) Basal ration + cholesterol 0.5% + DA extract 3.6 g/100 g (KE2), 5) Basal ration with 15% DA flour + 0.5% cholesterol (KT1) and 6) Basal ration with 30% DA flour + 0.5% cholesterol (KT2). The Total cholesterol, HDL, LDL cholesterol in serum were analysed at baseline, days 28, days 56 and at the end of study.

Results: The administration of high cholesterol (1%) ration increased blood lipid levels by 16 fold compared to that of control. The administration of 15% and 30% of DA flour could maintain blood lipid profile to normal condition, in particular at 30% substitution DA flour. However the water extract of DA can not maintain a normal blood lipids of high cholesterol treated rabbits

Conclusion: *Dioscorea alata* flour has suggested to have anti-hyperlipidemia effect. (*Health Science Indones 2014;1:23-9*)

Key words: *Dioscorea alata*, hyperlipidemia, atherosclerosis

Hyperlipidemia is one risk factor for atherosclerosis.¹ Epidemiology and experimental researches showed that food containing high cholesterol is highly related with hyperlipidemia condition. High lipid level, particularly Low Density Lipoprotein-Cholesterol (LDL-Cholesterol) showed significant relationship with the development of atherosclerotic plaque.² Many researches related to atherosclerosis were conducted to see the effect of bioactive compounds in normalizing hyperlipidemia and preventing the oxidation of blood lipid cholesterol. Currently, natural materials become a topic of interest of research as a potential substance producing biologically active component such as antioxidant, anti-hyperlipidemia or as anti-inflammatory substances. Tuber of *Dioscorea alata* (DA) has the potential to reduce the risk of cardiovascular disease with functional compounds.

The functional components of this tuber are anthocyanins, dietary fiber and diosgenin. Anthocyanins belongs to the polyphenolic compound which has antioxidant capacity by scavenging free radicals.³ Current research indicates that anthocyanins have physiological advantages as an antioxidant, anti-inflammatory, blood vessel relaxation, and as stabilizing compounds of capillaries.⁴ Anthocyanin could also improve blood lipid profile by increasing High Density Lipoprotein (HDL) and lowering Low Density Lipoprotein (LDL) plasma.⁵ Musilases are compound in DA tuber that contain complex carbohydrates and protein. The carbohydrate are mainly a dietary fiber consisting of hexose and pentose monomers. Musilases could regulate lipid metabolism with anti-hyperlipidemia effect.⁶ Diosgenin is a plant steroid that has anti-hyperlipidemia effect by suppressing cholesterol absorption and increased secretion of cholesterol in bile acid,⁷ therefore increased fecal cholesterol excretion.^{8,9}

With the potency, it is expected that supplementation of DA extract or flour could normalized the blood lipid profile of rabbits. This reserach is aimed to determine the efect of supplementation of DA extract and flour on blood lipid profile of rabbits fed with high cholesterol diet.

METHODS

Material and equipment

The material used in this study are: DA tuber flour, aquadest, male New Zealand White rabbits 5 months old, that obtained from the Indonesian Research Institute for Animal Production in Ciawi, Bogor. Rabbit basal ration and experimental ration, made in

Indofeed livestock feed in Bogor. Pure cholesterol, reagent for cholesterol, LDL, HDL and triglicerides analysis (Fluitest-Chol kit) were obtained from *Sigma* and *Analyticon Biotechnologies*. The equipment are shakers, centrifuge, freeze drier, spectrophotometer, a set of rabbits growing equipment, and blood taking equipments,

The production of ration

Rations was formulated base on nutritional need for normal growth of adult rabbits.¹⁰ Material having polyphenolic compound other than DA tuber did not included in the ration component. Ration composition calculation were performed using the WUFFDA (Windows-Based User Friendly Feed Formulation Workbook) Version 3 to obtain isocaloric and isoprotein rations.¹¹

0.5 g of pure cholesterol was mixed with 19.5 g of basal ration, to produce a small ration of 20 g. The rabbits in cholesterol groups (KE1, KE2, KT1 and KT2), were fed with the small ration containing cholesterol in advance, and after the small ration was consumed, 80 g of basal ration was added. Therefore total cholesterol given is 0.5 g in 100 g of basal ration/ day or 0.5% in the diet.

DA dried extract of maltodextrin, contained 25 g of anthocyanins per 100 g, whereas DA flour contained 1.4 g of anthocyanins per 100 g, or there was as much as 0.23 anthocyanins/15 g DA flour or 0.45 g anthocyanin/ 30 g DA flour. To get an equivalent amount of anthocyanin presented in 15 and 30 g DA flour, 0.9 and 1.8 g of maltodextrin extract were weight. This maltodextrin extracts was mixed with cholesterol ration, and this mixture was added by basal ration until the total weight is 20 g. This ration is given to the KE1 and KE2 group to be eaten in advance, then 80 of basal ration is added. Therefore the total ration given to these groups was 0.5 g of cholesterol plus 1.8 or 3.6 g anthocyanins extract in 100 basal ration/day.

The DA flour 15 and 30 ration were made by adding 150 g and 300 g DA flour into/ Kg of basal ration ingredients. All ingredients were processed into pellets, so that at the end, the ration contained 15 and 30% DA flour. These ration were fed to rabbits in KT1 and KT2 groups. Proximate content of the ration were analysed using AOAC methods. The content of dietary fiber was analysed using dietary fiber kit (Sigma-Aldrich, USA) using a combination of enzymatic and gravimetric methods.

***In vivo* experiment in rabbits**

In vivo experiment was conducted using 5 months-old male of New Zealand white rabbits with initial weight approximately 2.7 kg in each. The number of rabbits in each group was calculated based on the mean difference formula.¹³ To meet sampling size, the number of rabbits required in each group should be five. Rabbits were weighed and grouped according to the type of ration as follow.

- K1 = Control negative, Basal Ration
- K2 = Control positive cholesterol, Basal Ration + 0.5% cholesterol
- KE1 = Basal Ration + anthocyanins extract equal to 15 g DA flour (1.8 g) + 0.5% cholesterol
- KE2 = Basal Ration + anthocyanins extract equal to 30 g DA flour (3.6 g) + 0.5% cholesterol
- KT1 = Basal Ration contain 15% DA flour + 0.5% cholesterol
- KT2 = Basal Ration contain 30% DA flour + 0.5% cholesterol

The rabbits were adapted to the maintenance environment for 1 month. In adaptation phase, the standard ration was substituted gradually from 0% to 100% treatment ration. After one month, the rabbits were ready to eat treatment ration. During the study, the measurements of observed variable was carried out regularly. Rabbits was weight once a week, ration consumption was weighed and calculated everyday, blood lipids (total kolesterol, LDL, HDL, dan trigliserida) were measured at baseline, day 28, 56 and at the end of study.

Blood was taken from vein at the ears using butterfly sterile syringe. To obtain serum, blood was centrifuged at 100 g for 15 min at 4°C. Triacylglycerol (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-cholesterol), high-density lipoprotein cholesterol (HDL-cholesterol), were analyzed using commercial kits (Fluitest® Chol, HDL-Chol, LDL-Chol dan Triacylglycerol, Analyticon Biotechnologies).

The study design was a completely randomised design with five replications. Data were analyzed using analysis of variant (ANOVA) and Least significant different (LSD) analysis to determine the difference between treatments. The value was suggested to be significantly different if the P value is smaller than 0.05. All data presented in the form of mean ± standard deviation, The ethical approval was obtained from the Research Ethics Board of National Institute of Health Research and Development, Ministry of Health of Indonesia.

RESULTS

The compositions of rabbit ration are listed in Tabel 4.1. The macro nutrient of each ingredient is analysed, and the values are used as the basis for formulation (Tabel 4.2). The purpose of the formulation is to obtain rations that contain uniform number of calories, protein, fat and crude fiber, and comply with the required nutrient for normal growth of rabbits. According to Lebas (1997), nutrition requirements for a rabbit are 350-370 kcal/100 g of energy, 3-5 % of fat, and 15-18 % of protein, whereas the need of fiber was 10-14 %.

Rations are formulated to contain 0 %, 15 % and 30 % of DA flour. Formulations should meet nutritional and fiber needs for normal growth of rabbits. Protein, fat and fiber are ranged from 16.01-16.34 %, 3.2-4.92 %, and 10.51-11.84 %, while the energy is ranged between 360-366 kcal/100 g ration. With this composition, ration has meet the nutritional needs of a normal growth of adult rabbits. From the analysis, it can be seen that the content of protein, fat, crude fiber in the ration of all experimental groups are equivalent. However, the dietary fiber content of the basal ration, DA-15 ration and DA-30 were different.

Rabbits in each treatment group consumed the same amount of ration ($P = 0.3818$, $\alpha = 0.05$). The Number of ration consumed in 60 days was between 5106.9 - 5826.2 g or 83.72 -95.51 g / day. With a number of these rations, protein and calorie intake for each group ranged between 817.61 - 939.04 g or 13:40 to 15:39 g / day, and between 18.691 to 21.324 kcal or 306-349 kcal / day. The amount of energy and protein consumed by rabbits is an important factor to ensure that the difference in the parameters is caused by differences in treatment, and not due to differences in the amount of feed intake.

The differences in ration composition did not cause a significant different in weight of rabbits between groups, because the content of macro nutrient in each group was equal. Initial weight of rabbits were varied between 2255.7 - 2457.0 g (not significantly different, $P = 0.9961$, $\alpha = 0.05$), and the final weight were between 2987.0 - 3339.8 g. The weight gain is about 800 g/individual rabbit in 60 days, or about 90 g/individu/week.

The amount of cholesterol and anthocyanins consumed by the rabbits were calculated by subtracting the amount of given ration with the left behind. The amount of cholesterol consumed by a rabbit in 60 days is between 33.64 - 34.63 g or 0.50 to 0.57 g/

day. Analysis of variance showed that there was no significant difference in the amount of cholesterol consumed ($P = 0.2622$, $\alpha = 0.05$ level). The intake of anthocyanin extract between groups KE1 (extract 15) and KT1 (flour 15) or between groups extract and flour KE2 and KT2 is designed the same. To extract the DA 15 and DA 15 flour, the average intake 0.24 and 0.19 g / day, and to extract DA-30 and DA-30 starch intake the average was 0.45 and 0.40 g / day.

During the research, there were four times of blood lipids measurements. At the baseline, all of blood lipid parameters were in normal condition, where total cholesterol is between 54.03 - 89.88 mg / dL, HDL is between 29.65 - 41.43 mg / dL, LDL is between 43.03 - 60.05 mg / dL, and triglycerides is between 40.35 - 67.63 mg / dL. With this range, the difference in blood lipid components at baseline was not significant ($p = 0.5740$, $\alpha = 0.05$).

Table 1. The changing of blood total cholesterol level

Groups	Total cholesterol level (mg/dL)							
	Baseline		Day-28		Day-56		End	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
K0	73.53 ±	22.93	65.83 ±	21.13 ^c	56.35 ±	28.65 ^b	54.00 ±	6.35 ^c
K1	89.88 ±	41.66	1443.00 ±	446.84 ^a	2140.50 ±	904.97 ^a	1987.20 ±	770.66 ^a
KE1	70.45 ±	28.18	1424.50 ±	375.42 ^a	2152.00 ±	1133.90 ^a	1572.80 ±	218.21 ^{ab}
KE2	68.25 ±	21.21	1410.30 ±	320.30 ^a	2081.80 ±	701.98 ^a	2000.00 ±	920.13 ^a
KT1	54.03 ±	13.84	537.65 ±	304.08 ^b	680.35 ±	228.12 ^b	645.40 ±	228.94 ^{bc}
KT2	68.25 ±	19.13	250.55 ±	110.81 ^{bc}	438.80 ±	150.09 ^b	314.15 ±	150.71 ^c

*The value followed by different letter in the same column indicated a significant difference ($P \leq 0.05$)

Table 2. The changing of blood LDL cholesterol level

Groups	LDL cholesterol level (mg/dL)							
	Baseline		Day-28		Day-56		End	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
K0	47.55 ±	12.45	51.45 ±	14.52 ^c	48.50 ±	24.13 ^b	42.03 ±	7.81 ^b
K1	60.05 ±	23.31	1409.30 ±	185.54 ^a	978.50 ±	182.24 ^a	514.48 ±	230.31 ^a
KE1	43.18 ±	9.66	808.25 ±	720.77 ^b	974.50 ±	164.53 ^a	625.73 ±	346.88 ^a
KE2	43.03 ±	5.38	1348.00 ±	151.51 ^a	872.25 ±	339.61 ^a	761.27 ±	171.01 ^a
KT1	48.98 ±	17.39	208.77 ±	56.91 ^c	149.82 ±	12.20 ^b	207.05 ±	65.97 ^b
KT2	45.90 ±	17.32	157.10 ±	21.65 ^c	153.95 ±	17.91 ^b	109.58 ±	44.28 ^b

*The value followed by different letter in the same column indicated a significant difference ($p \leq 0.05$)

Table 3. The changing of blood HDL cholesterol level

Groups	HDL cholesterol level (mg/dL)							
	Baseline		Day-28		Day-56		End	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
K0	38.03 ±	12.69	33.83 ±	7.70 ^{ab}	25.03 ±	9.01 ^a	25.03 ±	9.86 ^a
K1	31.98 ±	13.25	27.95 ±	2.47 ^b	17.73 ±	5.34 ^a	17.73 ±	5.81 ^a
KE1	39.03 ±	16.64	42.30 ±	13.13 ^{ab}	24.30 ±	11.15 ^a	24.30 ±	7.82 ^a
KE2	35.25 ±	5.17	40.45 ±	11.15 ^{ab}	23.28 ±	8.69 ^a	23.28 ±	12.15 ^a
KT1	29.65 ±	6.42	36.13 ±	7.54 ^{ab}	20.68 ±	6.67 ^a	20.68 ±	3.04 ^a
KT2	41.43 ±	13.22	41.83 ±	8.87 ^a	23.70 ±	6.36 ^a	23.70 ±	10.17 ^a

*The value followed by different letter in the same column indicated a significant difference ($P \leq 0.05$)

Table 4. The changing of blood triacylglyceride level

Groups	Tryacylglyceride level (mg/dL)							
	Baseline		Day-28		Day-56		End	
K0	52.58 ±	11.62	83.00 ±	13.52 ^b	55.43 ±	20.07 ^c	68.38 ±	5.90 ^c
K1	57.08 ±	6.82	216.22 ±	94.34 ^a	186.28 ±	25.70 ^b	271.30 ±	68.10 ^a
KE1	51.53 ±	16.55	183.30 ±	28.83 ^a	203.60 ±	43.09 ^b	243.42 ±	42.29 ^a
KE2	61.13 ±	9.76	187.90 ±	26.42 ^a	251.42 ±	38.60 ^a	276.90 ±	35.33 ^a
KT1	48.25 ±	5.70	94.30 ±	20.81 ^b	91.80 ±	36.77 ^c	176.10 ±	27.02 ^b
KT2	47.20 ±	16.53	78.13 ±	16.97 ^b	68.33 ±	12.87 ^c	75.40 ±	22.27 ^c

*The value followed by different letter in the same column indicated a significant difference ($P \leq 0.05$)

DISCUSSION

Administration of cholesterol in rabbits led to a noticeable increase in total cholesterol, LDL-cholesterol, and triacylglycerol. These results are consistent with previous studies,^{14,15,16} which found that the lipid foods correlates with blood lipid profile of rabbits. Rabbits in particular, are very sensitive to dietary cholesterol, a normal rabbit will synthesize and secrete about 100 mg of cholesterol per day and maintain blood cholesterol concentrations at a maximum of 100 mg / dl, but the provision of cholesterol in the diet, will increase in the extreme concentration of cholesterol in the blood rabbit.¹⁰ Total cholesterol, LDL cholesterol and blood triacylglycerol in rabbits fed a high cholesterol diet (K1) showed a very high increase. On the measurement at day 28, it is known that the level of total cholesterol increased by 16-fold, and total and LDL cholesterol increased by 20-fold from the initial conditions (Table 1 and 2). These conditions could happen, because rabbits are herbivores that are very sensitive to dietary cholesterol, however the animal is not able to degrade or secrete cholesterol in the form of bile acids in sufficient quantities, to balance with what has been absorbed, so that the severe hyperlipidemia condition was occurred (Weisbroth 1974).¹⁷ The conditions of high levels cholesterol, LDL cholesterol and triacylglycerol is continuously increase until the 56th day of measurement, and a there was a slight decrease in the measurement at day 60.

The administration of cholesterol ration does not cause a significant differences in HDL-cholesterol levels between treatment groups (Table 4.6.). HDL has a function to bring the non-esterified cholesterol from the cells and from other accumulated lipoproteins, to the liver for bile acids secretion.² Low levels of HDL-cholesterol indicates a low cholesterol carried to the

liver to balance what has been absorbed. Therefore, the levels of cholesterol in the blood is very high.

The administration of water extract of DA (KE1 and KE2) to rabbits fed with high cholesterol diet can not maintain blood lipid profile in normal conditions. Levels of total cholesterol, LDL-cholesterol, and blood triacylglyceride of rabbits in these group are closer to K1 cholesterol group ($p = 0.000$, $\alpha = 0.05$ level). Water extract of DA tubers can not normalize blood lipid profiles of rabbits. Water extract of DA tubers contained anthocyanins which have the effect of lowering cholesterol absorption by modulating the withdrawal of cholesterol from the body to the liver, and secreted into bile acid form, through the activation of receptors that regulate the withdrawal.¹⁸ However, this modulation effect is not shown by giving 0.23 g and 0.45 g anthocyanin extract per day. This may be due, the insufficient dose which can lead the modulation effect.

Substitution 15 and 30% DA flour (KT-1 and KT-2) into ration, can maintain total blood cholesterol of rabbits to normal, especially in rabbits fed with 30% DA flour substitution. Rabbit blood cholesterol levels in both groups were not significantly different from those of normal control group (K0). Substitution of DA flour into rations not only provide anthocyanin compounds, but also dietary fiber, such as cellulose, lignin, hemicellulose and pectin.¹⁹ The results of the analysis indicate that the soluble fiber in the basal ration with no DA flour substitution was 6.13%, while the ration with 15% and 30% DA flour substitution contain 9.17% and 13% of dietary fiber respectively.

There are several mechanisms of dietary fiber in lowering serum cholesterol levels. The most likely mechanism is that the presence of dietary fiber

increases the excretion of bile acids. Soluble fiber lowers plasma cholesterol through its ability to bind bile acids in the gastrointestinal tract. Because soluble fiber binds bile acids, the formation of micelles become distracted and reabsorption of bile acids also decrease. This case will increase the excretion of the dietary fiberbile acid complex in the feces. Since the bile acids decreased, the replacement is carry out in two ways. Firstly, with taking more cholesterol from the liver and using it for the synthesis of bile acids, and not circulated to the body as VLDL (very low density lipoprotein). Secondly, the need of hepatic cholesterol will improve the regulation, synthesis, and LDL receptor activity, causing VLDL and LDL remnant are taken from the circulation in the body. The overall effect of these changes is a reduction in levels of LDL and total cholesterol serum.²⁰ In addition, the availability of dietary fiber also causes changes in the activity of LDL receptors. A study showed that the experimental animals fed with a high-fat diet, showed a low LDL receptor activity. However, if the high-fat diet and high cholesterol are given simultaneously with soluble fiber, thereforen the LDL receptor function will revert to normal.²¹

Another thing that is expected to play a role in lowering cholesterol is the diosgenin content in DA flour which belong to steroidal saponin grup. A study reported that plant saponins are proven to prevent the absorption of cholesterol in the lumen of the small intestine of animal models, therefore can decrease the concentration of plasma cholesterol.²² Diosgenin also been shown to increase the excretion of cholesterol through feces and increase the secretion of cholesterol through bile acid.⁸ Plant sterols and steroids have the hypocholesterolemia effect by inhibiting the absorption of dietary cholesterol and endogenous cholesterol. The existence of sterols and steroids from food will compete with cholesterol for micellar solubility, thus lowering the amount of cholesterol that is absorbed by the intestinal mucosal cells.²³

CONCLUSION

The supplementation of DA flour DA by 15% and 30% maintain the blood lipid profile of rabbits towards normal conditions, in particular at 30% substitution DA flour. However the water extract of DA can not maintain a normal blood lipids of high cholesterol treated rabbits.

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