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Blood Lipid Profile of Hypercholesterolemia *Rattus norvegicus L.* Fed with Sausages Containing Omega 3 and Omega 6 Fatty Acids

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ABSTRACT: The experiment was conducted to investigate blood lipid profile of hypercholesterolemia Rattus norvegicus L. fed with sausages containing omega 3 and omega 6 fatty acids. Twenty four male *Sprague dawley* breed rats of two months old were used in this experiment and placed in individual cage. They were grouped into four groups consisted of six rats and fed with basal ration, and drinking water ad lib. The rats were given sausages with different level of omega 3 and omega 6 fatty acids with ratio of 1 : 5.29. The levels were 1.78 g/rat/day (low level); 3.56 g/rat/day (medium level); and 7.11 g/rat/day (high level); and control. Blood was taken at the venous sinus retro-orbital of rats for the lipid profile analysis (cholesterol, triglyceride, low density lipoprotein, and high density lipoprotein). The data of lipid profile were analyzed statistically using analysis of variance and the differences between means were tested by Duncan's new multiple range test. The feeding of sausages containing omega 3 and omega 6 influenced significantly on the blood lipid profile of rats ($P<0.05$). The blood of rats fed with sausages containing omega 3 and omega 6 fatty acids at the level of 7.11 g/rat/day (high level) had lower cholesterol 113.77±5.65 mg/dl, triglyceride 81.55±7.35 mg/dl, and low density lipoprotein 50.56±6.41 mg/dl, and had higher high density lipoprotein 64.51±3.43 mg/dl compared to control that contained cholesterol 218.54±6.51 mg/dl, triglyceride 142.71±9.27 mg/dl, and low density lipoprotein 100.41±5.97 mg/dl, and high density lipoprotein 20.98±2.36 mg/dl ($P<0.05$). In conclusion, the rats fed with sausages containing omega 3 and omega 6 fatty acids at low, medium, and high levels improve the blood profile of rats in term of decrease of cholesterol, triglyceride, and low density lipoprotein and of increase of high density lipoprotein.

Keywords: Sausages, Lipid profile, Blood, *Rattus norvegicus* L., Hypercholesterolemia

INTRODUCTION

The degenerative diseases such as coronary heart disease and stroke were increased. The increasing of degenerative diseases mostly caused by consuming fast food or foodstuffs from animal origin. Meat derived from livestock slaughtered in Indonesia has low quality. This is due to the origin of livestock that used as draught cattle, so that the meat yielded contains a lot of saturated fatty acid and cholesterol (Setiyono, 2008). The consumption of meat containing a lot of saturated fatty acids and cholesterol is related to negative impact on health or significant health problems, including coronary heart disease and stroke (Krummel, 2008). Setiyono, (2008) reported that the Ongole cross breed meat fattening by feedlots contained fat 5.98% (Longissimus dorsi muscle) and 5.87% (Biceps femoris muscle) and contained cholesterol 81.39 mg/100 g of meat (Longissimus dorsi muscle) and 83.09 mg/100 g of meat (Biceps femoris muscle). According to Singapore General Hospital in Muharrami (2011) and Anonymous (2015[a]) the normal blood lipid profile contained cholesterol < 200 mg/dl, low density lipoprotein (LDL) < 130 mg/dl, high-

density lipoprotein (HDL) > 40 mg/dl, and triglycerides < 200 mg/dl. The bloods which contain cholesterol higher than 200 mg/dl is called hypercholesterolemia and related to atherosclerosis.

There are various prevention efforts of hypercholesterolemia. One of the prevention effort could be conducted by consuming healthy meat product such as sausages that contained omega 3 and omega 6 fatty acids. The food containing omega 3 and omega 6 fatty acids with the ideal ratio could overcome the cholesterol problems in the blood (Setiyono, 2008; Elmadfa and Kornsteiner, 2009; Anonymous, 2015[b]). The feeding of sausages containing omega 3 and omega 6 fatty acids from animal and plant such as cod liver oil and corn oil could reduce the cholesterol content in the blood. Therefore, the biological test on *Rattus norvegicus* L. needs to be conducted to prove that the sausages could reduce the cholesterol content in the blood of rats. The experiment was conducted to investigate the blood lipid profile of hypercholesterolemia *Rattus norvegicus* L. fed with sausages containing omega 3 and omega 6 fatty acids with ratio of 1 : 5.29.

MATERIALS AND METHODS

The materials used in the experiment consisted of twenty four male *Sprague dawley* breed rats of two months old (we used the *Sprague dawley* breed rats in this experiment because of the rats have a good responses when used as a subject in the experiment using cholesterol as an indicator (Fox *et al.*, 1984)), individual cage, basal ration/AIN-93-M (Reeves, 1997), lard/pork fat, sausages containing omega 3 and omega 6 fatty acids with ratio of 1: 5.29 at 11.37% fat content of sausages), reagents and kits used for analysis of total cholesterol and triglycerides, and reagents and kits used for analysis of HDL cholesterol. Reagent to measure total cholesterol containing phosphate buffer, 4-aminophenazone, phenol, peroxidase, cholesterol esterase, and cholesterol oxidase. Reagent to measure triglycerides containing phosphate buffer, 4-chlorophenol, ATP, Mg^{2+}, glycerokinase, peroxidase, lipoprotein lipase, 4-aminophenazone, and glycerol-3-phosphate oxidase, while the reagents used to measure HDL cholesterol containing N,N-bis (4-sulfobutyl)-m-toluidine disodium salt (DSBmT), cholesterol oxidase, peroxidase, 4-aminoantipyrine, cholesterol esterase, and detergents.

This experiment was conducted for 35 days and divided into three stages of experiment. In the first stage, twenty four male *Sprague dawley* breed rats of two months old were fed with basal ration/AIN-93-M and drinking water ad lib for 7 days. In the second stage, they were fed with lard 4 g/rat/day until hypercholesterolemia/blood cholesterol > 200 mg/dl for 14 days. In the third stage, they were grouped into four groups consisted of six rats. The groups were: P1(control); P2 (basal ration/AIN-93-M + sausages 1.78 g/rat/day); P3 (basal ration/AIN-93-M + sausages 3.56 g/rat/day); and P4 (basal ration/AIN-93-M + sausages 7.11 g/rat/day). The treatment was conducted for 14 days. Blood was taken at the end of each stage for the lipid profile analysis including cholesterol, triglyceride, low density lipoprotein (LDL), and high density lipoprotein (HDL). Blood was taken by using microcapiler hematocrit at the venous sinus retro-orbital of rats (Hrapkiewicz *et al*., 1998). Total cholesterol and HDL cholesterol were analyzed by using CHOD-PAP (cholesterol oxidase-p-aminophenazone) method. Triglyceride were analyzed using GPO-PAP (glycerol-3-phosphate oxidase-p-aminophenazone) method (Rodriguez *et al*., 2000). LDL cholesterol were calculated mathematically (LDL cholesterol (mg/dl) = (total cholesterol (mg/dl) – HDL cholesterol (mg/dl)) – (triglycerides (mg/dl) / 5) (Ginsberg *et al*., 1998). The data of lipid profile were analyzed statistically using analysis of variance and the differences between means were tested by Duncan's new multiple range test (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Table 1. Blood lipid profile of *Rattus norvegicus* L. fed with sausages containing omega 3 and omega 6 fatty acids with ratio 1 : 5.29 at low, medium, and high levels

Variable	Feeding sausages/sausages levels			
	Control	1.78 g/rat/day	3.56 g/rat/day	7.11 g/rat/day
Cholesterol (mg/dl)				
First stagens	103.83±2.61	100.00±3.38	102.09±3.91	99.54±3.04
Second stagens	218.87±6.26	214.58±4.63	213.00±5.70	217.63±5.99
Third stage	218.54±6.51^{d}	159.16±4.45^{c}	128.67±5.23^{b}	113.77±5.65^{a}
LDL (mg/dl)				
First stage	42.63±4.19^{b}	37.87±3.49^{a}	39.34±2.81^{ab}	36.39±2.61^{a}
Second stagens	100.30±5.86	96.15±4.49	95.24±5.39	99.70±5.98
Third stage	100.41±5.97^{d}	76.93±4.17^{c}	64.31±4.98^{b}	50.56±6.41^{a}
HDL (mg/dl)				
First stagens	75.73±3.14	72.65±3.97	74.87±4.20	71.62±4.37
Second stagens	23.29±2.69	28.38±5.11	28.68±4.26	25.91±3.85
Third stage	20.98±2.36^{a}	35.20±4.02^{b}	48.85±4.21^{c}	64.51±3.43^{d}
Triglycerides (mg/dl)				
First stage	72.08±5.91^{b}	64.53±3.00^{a}	69.37±3.39^{ab}	64.96±2.96^{a}
Second stagens	141.33±9.33	135.90±6.23	133.64±7.61	139.22±8.12
Third stage	142.71±9.27^{d}	108.33±5.35^{c}	98.07±6.69^{b}	81.55±7.35^{a}

^{abcd}Superscripts at the same row indicate significant differences (P<0.05)

The data of blood lipid profile were shown at Table 1. The feeding of sausages containing omega 3 and omega 6 influenced significantly on the blood lipid profile of rats (P<0.05). The feeding of sausages containing omega 3 and omega 6 fatty acids with ratio of 1 : 5.29 at low, medium, and high levels decreased cholesterol, triglyceride, and low density lipoprotein (LDL) but it increased high density lipoprotein (HDL). The blood of rats fed with sausages containing omega 3 and omega 6 fatty acids at the level of 1.78 g/rat/day (low level) contained cholesterol 159.16±4.45 mg/dl, triglyceride 108.33±5.35 mg/dl, LDL 76.93±4.17 mg/dl, and HDL 35.20±4.02 mg/dl. The blood of rats fed with sausages containing omega 3 and omega 6 fatty acids at the level of 3.56 g/rat/day (medium level) contained cholesterol 128.67±5.23 mg/dl, triglyceride 98.07±6.69 mg/dl, LDL 64.31±4.98 mg/dl, and HDL 48.85±4.21 mg/dl. The blood of rats fed with sausages containing omega 3 and omega 6 fatty acids at the level of 7.11 g/rat/day (high level) contained cholesterol 113.77±5.65 mg/dl, triglyceride 81.55±7.35 mg/dl, LDL 50.56±6.41 mg/dl, and HDL 64.51±3.43 mg/dl. The blood of rats fed with sausages containing omega 3 and omega 6 fatty acids at the level of 1.78 g/rat/day (low level); 3.56 g/rat/day (medium level); and 7.11 g/rat/day (high level) had lower cholesterol, triglyceride, and LDL, and had higher HDL compared to control that contained cholesterol 218.54±6.51 mg/dl, triglyceride 142.71±9.27 mg/dl, LDL 100.41±5.97 mg/dl, and HDL 20.98±2.36 mg/dl. The blood lipid profile of rats fed with sausages containing omega 3 and omega 6 fatty acids was in agreement with the Singapore General Hospital in Muharrami (2011) and Anonymous (2015[a]) regarding to their blood lipid profile which contained cholesterol < 200 mg/dl, low density lipoprotein (LDL) < 130 mg/dl, high-density lipoprotein (HDL) > 40 mg/dl, and triglycerides < 200 mg/dl.

Omega 3 and omega 6 fatty acids with the ideal ratio affected low density lipoprotein (LDL) receptors, decreased very low density lipoprotein (VLDL), decreased apolipoprotein B as the component of low density lipoprotein (LDL), and increased apolipoprotein A1 as the component of high density lipoprotein (HDL) therefore blood LDL of rats decreased while blood HDL of rats increased. HDL could decrease blood cholesterol because of the HDL function in the blood which pick up cholesterol from tissues to the liver then degraded or converted to bile acids (Assmann and Schulte, 1992; Mayes, 1996; Pastore, 2003; Setiyono, 2008; Elmadfa and Kornsteiner, 2009; Siri-Tarino *et al*., 2010) therefore the feeding of sausages containing omega 3 and omega 6 with the ideal ratio on rats decreased blood cholesterol of rats because the cholesterol was used as the component of bile acids production. Omega 3 and omega 6 fatty acids with the ideal ratio also affected the activity of the lipoprotein lipase enzyme. The lipoprotein lipase enzyme degraded triglycerides in chylomicrons and VLDL into glycerol and fatty acids (Assmann and Schulte, 1992; Mayes, 1996; Pastore, 2003; Setiyono, 2008; Elmadfa and Kornsteiner, 2009; Siri-Tarino *et al*., 2010) therefore the increase of lipoprotein lipase enzyme activity could decrease blood triglycerides of rats. LDL is the metabolic products of VLDL, therefore decrease of the VLDL because the feeding of sausages containing omega 3 and omega 6 with the ideal ratio on rats could decrease blood LDL of rats. Linolenic/α-linolenic fatty acid (omega 3 fatty acid) converted into eicosa pentaenoic acid (EPA) and docosa hexaenoic acid (DHA) and linoleic fatty acid (omega 6 fatty acid) converted into arachidonic fatty acid (Anonymous, 2013). EPA and DHA could reduce VLDL, inhibit thromboxane production, increase prostacyclin, lowering blood viscosity, and prevents thrombosis (Soerjodibroto, 2005). EPA and DHA also could be lowering blood triglycerides in individuals with hypertriglyceride and prevent blood platelets.

CONCLUSION

The rats fed with sausages containing omega 3 and omega 6 fatty acids with ratio 1 : 5.29 at low, medium, and high levels improve the blood profile of rats in term of decrease of cholesterol, triglyceride, and low density lipoprotein (LDL) and of increase of high density lipoprotein (HDL).

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