

GOAT MILK KEFIR SUPPLEMENTED WITH PORANG GLUCOMANNAN IMPROVES LIPID PROFILE AND HAEMATOLOGICAL PARAMETER IN RAT FED HIGH FAT AND HIGH FRUCTOSE DIET

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Abstract

Background and Aims: Diet with a high fat and high sugar is associated with an increased incidence of the metabolic syndrome. Kefir has been known as a natural probiotic, while glucomannan from porang (*Amorphophallus oncophyllus*) tuber was demonstrated as prebiotic *in vivo*. Probiotics and prebiotics can be used adjuvant nutritional therapy for metabolic syndrome. The aim of this study was to evaluate the effect of goat milk kefir supplemented with porang glucomannan on the lipid profile and haematological parameters in rats fed with a high-fat/high-fructose (HFHF) diet.

Materials and methods: Rats were divided into 5 groups: normal diet; HFHF; HFHF + kefir; HFHF + kefir + glucomannan; and HFHF + simvastatin. **Results:** There were significant differences before and after treatment in triglycerides and total cholesterol in HFHF + kefir+glucomannan group. The HFHF rats administered kefir with or without glucomannan had higher levels of lymphocytes and lower neutrophils compared to HFHF group ($p < 0.05$). Only goat milk kefir without glucomannan proved to reduce platelets number. **Conclusion:** Goat milk kefir supplemented with porang glucomannan could improve the health of rats fed high-fat/high-fructose, by decreasing plasma triglycerides, total cholesterol, and their immunomodulatory effect by decreasing number of neutrophils and increasing the lymphocytes. Especially for goat milk kefir had antithrombotic activity which important to prevent cardiovascular diseases.

key words: goat milk kefir, porang glucomannan, lipid profile, haematological parameter.

Background and Aims

Recently, the consumption of goat milk and its derivatives is increasing more interest by consumers and producers due to their nutritional value and functional properties. Goat milk is

widely used to accelerate the patients healing due to the smaller fat globule and soft curd, thus it is easily digested and absorbed by the body. Fat globules in goat milk are smaller in diameter and the size distribution of globules has a larger proportion of smaller particles than in cow milk

("Natural homogenized"). The large number of fat globules with small diameter and higher contents of short- and medium-chain fatty acids makes the goat milk more digestible. This is because the total surface area of the globules is very effectively to contact with pancreatic lipase activity [1]. Goat milk also has lower α -s casein than cow milk, so that the curd is also softer which directly influence the digestibility in the gastrointestinal tract, cause the formation of smaller and less dense cluster in goat milk compared to cow milk. In addition, the unique characteristics of goat milk have been fairly good surveyed regarding nutritional value and health effects. The easier digestibility of goat milk, the appropriate composition of fatty acids, protein and its content of bioactive components seems to give properties suitable for treating or preventing certain medical conditions. The oligosaccharides of goat milk have beneficial effects on malabsorption disorders and inflammatory bowel diseases [2]. Recently, kefir was mentioned as potential source of probiotics and substances that very important for the health. Biologically, it acts as antioxidant, antitumor agent, antimicrobial and immunomodulator [3]. Water kefir was found to be cheaper product that demonstrated improvement in lipid profiles in diabetic rats even when consuming for short time [4].

Porang is local plant that grows in the tropical forest such as Indonesian that contains a lot of glucomannan which can be utilized as a gelling agent and water binding. Glucomannan is a hydrocolloid, composed of D-mannose and D-glucose in a ratio of 3:2 with β -(1, 4)-linkages with a low degree of acetyl groups [5]. According to Harmayani *et al.* [6], porang glucomannan has a potential as prebiotic that improve the balance of colon microbial. It has 86.43% solubility, 34.50% water binding capacity (WHC), 5400 cP viscosity, 9.4 degree

of polymerization, 13.7% degree of acetylation and 92.69% purity. A study [7] showed that glucomannan could reduce total cholesterol, LDL, triglyceride and/or increased HDL, and also decreased body weight gain.

In a previous study, esterified glucomannan that was supplemented in broiler diet exposed to aflatoxin, significantly improved on haematological parameters [8]. However, the effect of supplemented porang glucomannan in goat milk kefir has not been studied on individuals who are given a high fat and high fructose diet.

Modern lifestyle with a high consumption of high-fat and high-sugar may lead to an increased incidence of metabolic syndrome. Metabolic syndrome is a mix disorder caused by a group of interconnected factors that enhance the risk of cardiovascular diseases and type 2 diabetes. Obesity is the main precursor for metabolic syndrome that can be targeted in developing various therapies. Diet is one option for the management of obesity [9]. Therefore, it is needed to maintain the health through the proper diet, for example by consumption of functional foods. One of the functional food is a fermented milk product containing probiotics and/or prebiotics. The main purpose of this study was to evaluate the effect of goat milk kefir supplemented with glucomannan from porang tuber on lipid profile and haematological parameters in rats fed high-fat/ high-fructose diet.

Material and Methods

Extraction of glucomannan and kefir preparation

Porang tuber was obtained from the forest in Nglanggeran, Yogyakarta, Indonesia. Extraction of glucomannan was according to the cited reference [10] with modification. Goat milk was obtained from Ettawah Crossbred goat in Turi,

Sleman, Yogyakarta Indonesia. Whey protein concentrate (WPC) was given by Sari Husada Milk industry in Yogyakarta Indonesia. Kefir grain was obtained from local supplier in Yogyakarta. Goat milk kefir was prepared traditionally according to [11] with slight modification. Goat milk, 0.1% WPC, 0.3% porang glucomannan were mixed, pasteurized at 75°C for 15 min, and cooled at room temperature. Kefir grains (2%) were inoculated into pasteurized milk and incubated at room temperature for 18 h. After incubation, the kefir was filtered to separate kefir grain.

Experimental: animals

Male Sprague Dawley rats of 8-12 weeks old were divided into 5 groups (each group used 6 rats): 1) Normal control (negative control rats) that received standard diet only, 2) Rat fed high-fat/high-fructose (HFHF) (positive control), 3) Rat fed HFHF supplemented with kefir, 4) Rat fed HFHF supplemented with kefir +glucomannan, 5) Rat fed HFHF + simvastatin. Kefir in group 3 was added with 1% WPC, and kefir in group 4 was added with 1% WPC and 0.3% glucomannan. The dose of kefir was 3.6 mL/200 g body weight/ day, for 4 weeks. Before treatment, the rats were acclimated with standard diet AIN-93 for 1 week, and then fed high fat and high fructose for 2 weeks. The rats were then divided into 5 groups as above. High fat and high fructose diet were administered until the end of experiment (4 weeks). The composition of standard diet and high-fat/high-fructose diet were formulated according to [12] and [13]. According to [14], most patients achieve a decrease in cholesterol by using a recommended dose of simvastatin as much as 40 mg / day. If the dose for human was 40 mg, then the dose for rat was $0.018 \times 40 \text{ mg} = 0.72 \text{ mg}$.

All procedures related to animal experiment in this study were approved by Medical and

Health Research Ethics Committee (MHREC), Faculty of Medicine Universitas Gadjah Mada, Indonesia (Approval number: KE/FK/95/EC/2015).

Lipid profile analysis

Lipid profiles of blood plasma in rats were analyzed by enzymatic-photometric methods using Kit from DiaSys (Diagnostic Systems GmbH & Co) by Photometer (Merck-Microlab 300) at 540 nm. Total cholesterol and HDL cholesterol were measured by cholesterol oxidase-peroxidase-4 aminophenazone (CHOD-PAP) method. Triglyceride was measured by glycerol-3-phosphate oxidase, peroxidase and chromogenic reaction with 4-aminophenazone (GPO-PAP) method. HDL cholesterol was determined after lipoprotein precipitation with phosphotungstic acid and magnesium chloride, whereas LDL was calculated by a formula [15,16].

Haematological parameters analysis

Parameters of hematological in rats were analyzed by Automated Hematology Analyzer (Sysmex model KX-21, Japan). Blood samples were collected from the experimental rats at 0 and 28 day of the experiment. The haematological parameters analyzed were red blood cell (RBC), white blood cells (WBC) and platelets (PLT) parameters. Red blood cells parameters included hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell volume distribution width (RDW). White blood cells count included lymphocytes (LYM) and neutrophils (NEUT), whereas the platelet parameters mean platelet volume (MPV), platelet distributin width (PDW) and platelet-large cell ratio (P-LCR).

Statistical analysis

The variances of rat's body weight were analyzed by one-way ANOVA using SPSS version 17.0. The differences of lipid profiles and haematological parameters on rats before and after treatments (pre-and post-test) were analyzed by Paired Sample T-Test and differences between treatments in haematological parameters were analyzed by one-way ANOVA.

Results

Body weight

The results showed that the body weight of rats has a tendency to remain increased and the HFHF rat had highest value compared to other treatments. Before treatments, the body weight of rats varied, so that the statistical analysis was done for delta (difference in weight before and after treatments) of body weight. However, the results showed that the delta of body weight for all treatments were not significantly different ([Table 1](#)).

Table 1. The average of body weight in rats before and after various treatments.

Treatments	Body weight (g)		
	Before	After	Delta
Normal control	253.16±21.81 ^{ab}	305.66±18.14 ^b	52.50±21.36 ^a
HFHF	296.28±41.49 ^c	325.14±29.73 ^b	43.33±13.30 ^a
HFHF+kefir	275.85±26.74 ^{bc}	319.85±31.15 ^b	44.00±10.93 ^a
HFHF+kefir+glucomannan	269.50±21.23 ^{ab}	323.33±26.60 ^b	53.83±10.70 ^a
HFHF+simvastatin	237.14±24.61 ^a	275.00±26.10 ^a	37.85±9.04 ^a

^{abc}Different letters within the same column indicate significantly different ($p < 0.05$)

Table 2. Lipid profiles in rats before and after various treatments.

Treatments	Triglyceride (mg/dL)		Total cholesterol (mg/dL)		LDL (mg/dL)		HDL (mg/dL)	
	Before	After	Before	After	Before	After	Before	After
Normal control	125.65 ^a	54.73 ^a	73.25 ^a	53.50 ^a	34.00 ^a	18.60 ^b	1.41 ^a	20.13 ^b
HFHF	100.42 ^a	60.12 ^a	63.48 ^a	56.68 ^a	28.15 ^a	25.30 ^a	3.91 ^a	19.38 ^a
HFHF+kefir	130.62 ^a	95.19 ^a	55.51 ^a	48.97 ^a	22.74 ^a	16.54 ^a	4.94 ^a	18.34 ^a
HFHF+kefir+glucomannan	119.44 ^a	65.20 ^b	57.68 ^a	45.06 ^b	21.43 ^a	14.58 ^a	5.48 ^a	11.53 ^a
HFHF+simvastatin	201.10 ^a	105.34 ^a	63.40 ^a	49.30 ^b	23.02 ^a	17.40 ^a	5.06 ^a	17.30 ^b

Different letters within the same row indicate significantly different between before and after treatment ($p < 0.05$)

HFHF: High-fat high-fructose

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Plasma lipid profile

The results from [Table 2](#) showed that kefir supplemented with porang glucomannan has significant effect on triglyceride and total cholesterol ($p < 0.05$) in plasma of rats fed high-fat/high-fructose diet. Simvastatin also could reduce total cholesterol and increase HDL ($p < 0.05$) in rats fed high-fat and high-fructose.

Haematological parameters

Haematological parameters shown in Table 3-5, indicated that WBC, RBC, HGB, HCT, PLT, PDW, MPV and P-LCR pre-and post-test were significantly different ($p < 0.05$) for all treatments. For MCV, MCH, RDW in HFHF group, MCH in HFHF+kefir group, MCH, LYM and NEUT in kefir + glucomannan group, and RDW in simvastatin group also showed significantly different in pre- and post-test.

Parameters of red blood cells (RBC) in rats with various treatments are presented in [Table 3](#).

Table 3. The average of red blood cells parameters of rats in various treatments.

Red blood cell parameter	Pre-post	Normal control	HFHF	HFHF+Kefir	HFHF+Kefir+glucomannan	HFHF+Simvastatin
RBC ($\times 10^4/\mu\text{L}$)	Post	772.00 \pm 39.15 ^a	782.00 \pm 58.10 ^a	791.00 \pm 69.39 ^a	807.33 \pm 44.15 ^a	764.00 \pm 49.38 ^a
	Pre p*	146.60 \pm 14.84 0.000	150.28 \pm 13.00 0.000	140.42 \pm 18.36 0.000	142.16 \pm 12.65 0.000	135.71 \pm 24.85 0.000
HGB (g/dL)	Post	14.53 \pm 0.49 ^a	13.75 \pm 0.82 ^a	13.47 \pm 1.36 ^a	13.30 \pm 1.01 ^a	13.75 \pm 0.89 ^a
	Pre p*	6.13 \pm 5.21 0.013	2.78 \pm 0.24 0.000	2.64 \pm 0.37 0.000	2.73 \pm 0.18 0.000	2.57 \pm 0.45 0.000
HCT (%)	Post	43.28 \pm 2.01 ^a	41.05 \pm 2.41 ^a	41.68 \pm 2.63 ^a	41.60 \pm 4.54 ^a	41.60 \pm 4.54 ^a
	Pre p*	8.52 \pm 0.72 0.000	8.17 \pm 2.41 0.000	8.02 \pm 0.52 0.000	7.85 \pm 0.49 0.000	7.32 \pm 1.38 0.000
MCV (fL)	Post	56.08 \pm 0.65 ^a	52.55 \pm 1.53 ^b	52.35 \pm 2.95 ^b	51.50 \pm 4.14 ^b	53.17 \pm 2.23 ^a
	Pre p*	60.13 \pm 7.54 0.216	54.44 \pm 1.53 0.001	57.80 \pm 6.21 0.187	55.36 \pm 3.33 0.103	53.92 \pm 1.27 0.394
MCH(pg)	Post	18.85 \pm 0.51 ^a	17.62 \pm 0.63 ^{abc}	17.07 \pm 1.57 ^{bc}	16.50 \pm 1.40 ^c	18.05 \pm 1.49 ^{ab}
	Pre p*	18.68 \pm 0.54 0.654	18.54 \pm 0.79 0.006	18.81 \pm 0.73 0.007	19.26 \pm 0.97 0.002	19.00 \pm 0.72 0.188
MCHC (g/dL)	Post	33.61 \pm 0.58 ^a	33.52 \pm 0.45 ^a	32.27 \pm 2.09 ^a	32.10 \pm 1.60 ^a	33.91 \pm 1.88 ^a
	Pre p*	31.38 \pm 2.7 0.130	34.07 \pm 1.22 0.179	32.00 \pm 3.21 0.654	34.86 \pm 1.89 0.084	35.20 \pm 1.56 0.256
RDW (fL)	Post	30.60 \pm 0.75 ^a	33.42 \pm 4.06 ^{ab}	37.00 \pm 4.68 ^b	35.30 \pm 2.43 ^b	36.04 \pm 3.45 ^b
	Pre p*	35.51 \pm 11.38 0.343	28.38 \pm 0.70 0.015	32.23 \pm 4.25 0.144	33.64 \pm 6.73 0.632	28.94 \pm 1.16 0.004

*p < 0.05 values within each column indicate significantly different between pre- and post-test

^{abc}Different letters within the same row indicate significantly different (p<0.05)

HFHF: high-fat high-fructose

Table 4. The average of white blood cells parameters of rats in various treatments.

White blood cell parameters	Pre-post	Normal control	HFHF	HFHF+Kefir	HFHF+Kefir+glucomannan	HFHF+Simvastatin
WBC($\times 10^2/\mu\text{L}$)	Post	127.20 \pm 23.27 ^a	120.57 \pm 25.19 ^a	105.57 \pm 29.86 ^a	112.00 \pm 27.09 ^a	149.71 \pm 56.96 ^a
	Pre p*	16.00 \pm 3.16 0.000	18.50 \pm 4.54 0.000	20.14 \pm 5.72 0.000	20.16 \pm 3.97 0.001	24.71 \pm 6.23 0.001
LYM (%)	Post	72.71 \pm 3.97 ^a	60.60 \pm 14.05 ^b	70.58 \pm 8.18 ^a	73.88 \pm 4.53 ^a	75.68 \pm 2.38 ^a
	Pre p*	72.28 \pm 11.13 0.920	67.70 \pm 9.04 0.328	60.09 \pm 11.44 0.479	81.56 \pm 5.83 0.013	73.60 \pm 4.98 0.262
NEUT (%)	Post	26.48 \pm 4.78 ^a	39.40 \pm 14.83 ^b	29.41 \pm 8.18 ^a	26.11 \pm 4.53 ^a	24.31 \pm 2.38 ^a
	Pre p*	25.82 \pm 11.40 0.719	34.75 \pm 6.91 0.417	30.92 \pm 11.44 0.479	18.43 \pm 5.83 0.013	26.40 \pm 4.98 0.262

*p < 0.05 values within each column indicate significantly different between pre- and post-test

^{abc}Different letters within the same row indicate significantly different (p<0.05)

HFHF: high-fat high-fructose

Based on results from Table 3, in the post-test showed that kefir with and without porang glucomannan had no effect on RBC, HGB, HCT, MCHC in rats fed high-fat/high-fructose.

Parameter of white blood cells in rats with various treatments are presented in [Table 4](#). The rats supplemented with kefir had no effect on

WBC, but it could increase in LYM, and could decrease in NEUT of HFHF rats that was similar to the normal control rats. While LYM and NEUT of HFHF rats without kefir supplementation had a lower LYM and higher NEUT than the kefir supplemented rats (p<0.05) ([Table 4](#)).

Parameter of platelet in rats with various treatments is shown on [Table 5](#). There were no significant differences in PLT between normal

control rats and HFHF rats, whereas goat milk kefir without glucomannan supplementation in HFHF rats could decrease the PLT

Table 5. The average of platelet parameters of rats in various treatments.

Platelet Parameters	Pre-post	Normal control	HFHF	HFHF+Kefir	HFHF+Kefir+glucomannan	HFHF+Simvastatin
PLT (x104/ μ L)	Post	102.90 \pm 21.55 ^a	99.72 \pm 14.72 ^{ab}	70.81 \pm 30.22 ^c	81.93 \pm 29.57 ^{abc}	75.08 \pm 12.91 ^{bc}
	Pre p*	16.42 \pm 1.69 0.006	18.98 \pm 3.58 0.000	14.28 \pm 4.14 0.002	14.68 \pm 3.08 0.003	15.81 \pm 4.62 0.000
MPV (fL)	Post	6.53 \pm 0.22 ^a	6.44 \pm 0.17 ^a	6.51 \pm 0.42 ^a	6.53 \pm 0.48 ^a	6.62 \pm 0.22 ^a
	Pre p*	7.85 \pm 0.73 0.010	7.71 \pm 0.52 0.001	8.31 \pm 0.66 0.000	8.00 \pm 0.62 0.006	8.20 \pm 0.32 0.000
PDW (fL)	Post	7.81 \pm 0.49 ^a	7.77 \pm 0.31 ^a	8.20 \pm 0.89 ^a	8.08 \pm 0.86 ^a	8.31 \pm 0.53 ^a
	Pre p*	9.11 \pm 0.89 0.017	9.52 \pm 0.85 0.001	11.02 \pm 1.07 0.000	10.11 \pm 1.13 0.004	10.44 \pm 0.97 0.001
P-LCR (%)	Post	5.15 \pm 0.95 ^a	4.47 \pm 0.93 ^a	4.32 \pm 2.56 ^a	5.84 \pm 2.21 ^a	5.34 \pm 0.93 ^a
	Pre p*	11.96 \pm 5.94 0.045	10.04 \pm 2.58 0.003	14.11 \pm 5.07 0.001	11.62 \pm 3.63 0.012	13.57 \pm 2.54 0.000

*p < 0.05 values within each column indicate significantly different between pre- and post-test

^{abc}Different letters within the same row indicate significantly different (p<0.05)

HFHF: high-fat high-fructose

However, for all treatments there were no effects on MPV, PDW and P-LCR.

Discussion

Goat milk kefir with or without porang glucomannan have no effect on body weight of rats fed HFHF. This present study was similar to a previous study, that glucomannan supplementation had no effect on enhancing weight loss in overweight and medium obese individuals consuming self-selected diets and keeping regular physical activity patterns. However, glucomannan could increase weight loss when used in conjunction with either a normocaloric or a hypocaloric diet [11].

The effect of goat milk kefir supplemented with porang glucomannan in the present study was similar to a previous study that glucomannan decreased triglyceride and total cholesterol significantly but had no effect on HDL cholesterol [17]. Other previous study also showed that glucomannan isolated from *Eulophia herbacea* tuber had hypolipidemic activity [18].

Due to a decrease in plasma triglyceride and total cholesterol occurred in rats fed HFHF supplemented with kefir+glucomannan, so that the mechanism of reduction triglyceride and cholesterol involved probiotics in kefir and involved porang glucomannan. In a previous study showed that kefir fed in normal rats may decrease total cholesterol significantly [19]. The possible mechanisms of probiotics involved in the hypolipidemic effect may be the assimilation of cholesterol by bacterial growing cells; the binding of cholesterol to the bacterial cellular surface, thereby decreasing the absorption of cholesterol back into the body; the deconjugation of bile acids by bacterial acid hydrolyses, enhancing cholesterol excretion of deconjugated bile salts and enhancing cholesterol uptake and metabolism in the liver as compensatory reaction, since bile acids are formed from cholesterol in the liver; inhibition of hepatic cholesterol and triglyceride synthesis through the action of short chain fatty acids (SCFAs), especially propionic acid [20]. While the mechanism of glucomannan in the

hypolipidemic effect through its viscosity and fermentability [21]. Since glucomannan is highly viscous dietary fiber, thereby the increasing viscosity in intestinal contents and then decrease cholesterol absorption [22]. It has been known that human intestinal bacteria such as *Bifidobacterium adolescentis*, *Bacteroides thetaiotaomicron* and *Clostridium butyricum*-*Clostridium beijerinckii* group have enzymes that degrade glucomannan [23-25]. Furthermore, the SCFAs produced by cecal fermentation are possibly involved in lowering plasma cholesterol levels by excluding the counteractive induction of hepatic cholesterol synthesis caused by an increase in bile acid excretion [26].

The present study was similar to partly of a previous study that simvastatin significantly lowered total cholesterol, triglyceride, LDL and increased HDL cholesterol in rats induced with alloxan [27]. Statin, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor is inhibitor for cholesterol synthesis [28]. Statins may increase LDL receptor expression in liver, thereby reduced LDL level (increasing the LDL particle clearance) in blood through inhibition of cholesterol synthesis in liver [29].

The difference of haematological parameters before and after treatments was due to age differences of rats in pre-and post-test. According to [30], blood constituents change in relation to the physiological status of an animal. These changes were often caused by various factors; some of which were genetic and others, non-genetic. Age, sex, breed and management systems are among the factors that influence blood-based parameters of farm animals.

The effect of goat milk kefir on parameter of RBC in this study different with a previous study that fermented milk supplemented with probiotics (*Bifidobacterium lactis* Bi-07 and *Lactobacillus acidophilus* NCFM) and a prebiotic

(isomaltooligosaccharide) showed significantly different on HGB in rats compared with the control group [31]. However, the result of the present study was similar with a study by Rosa [19] that kefir had no effect on HCT in normal rats. The differences may be due to differences in probiotics or prebiotics type in kefir, animal condition in this study, and also fermented milk in the previous study.

Total number of WBC in the present study after treated with kefir was similar to a previous study by [32], that the total WBC (leukocyte) counts were not significantly altered after giving of *O. gratissimum* leaves extract, and also similar to a study by Rosa [19], that the total leukocytes remained unchanged in normal rats fed kefir.

In this study, the rats fed HFHF showed an increase in NEUT. In HFHF rats (positive control) may be sensitive to infection, so that NEUT increased. This indicates that there was a decrease in cellular immunity. Neutrophils act as the first-line of defense cells and the reduction of their functional activity contributes to the high susceptibility and severity of infections in diabetes mellitus. Clinical studies in diabetic patients and experimental studies in diabetic rats and mice clearly showed consistent defects of neutrophil chemotactic, phagocytic and microbicidal activities [33]. Despite their critical role in the protection from severe disease, the presence of polymorphonuclear (PMN) was correlated with haemorrhagic lesions, epithelial barrier permeability, and cellular inflammation in the lungs [34]. Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms [35].

The NEUT count in HFHF rats+ kefir that added with or without added glucomannan were the same as the normal control rats. This means

that kefir has a component that protect HFHF rats from infection. Kefir contains many microorganisms including several probiotics that produce bioactive components from microbial metabolic process that have health benefits such as antimicrobial, antitumor, anticarcinogenic and immunomodulatory activity [35,6]. Lactic acid resulted from kefir fermentation by *Lactobacillus kefiranofaciens*, *Candida kefir*, *Saccharomyces boluradii* can act as inhibitory agents through the toxicity due to membrane cells damage, cytosol acidification and anion accumulation intracellularly [37-39]. The present study was also similar to other study by [40], that rats aflatoxicosis had higher neutrophile and it decreased if glucomannan was added in feeding formulation.

Decreasing of LYM in rats fed HFHF, indicates that there was a decrease in cellular immunity. However, supplemented kefir with or without porang glucomannan could increase of blood lymphocyte in rats fed HFHF. This study was similar to a previous study by Abdelhady and El-Abasy [41], that rabbit fed prebiotic and probiotic and infected with *Pasteurella multocida* showed an increase the number of lymphocytes. According to Aboderin and Oyetayo [42], the lymphocyte counts of rats fed *Lactobacillus plantarum* isolated from fermenting corn slurry were higher than the control (without *L. plantarum*). Other study showed that oral ingestion of lactic acid bacteria by rats increases lymphocyte proliferation and interferon production [43]. Therefore, the lactic acid bacteria as above have immunostimulatory effect. Actually, supplementation of porang glucomannan in kefir fermentation in this study had the same effect with kefir without glucomannan on lymphocyte number. This is explained by [44] that there was no effect of oxidized konjac glucomannan supplementation in mice fed high-fat diet on haematological

parameters. However, other substances such as kefiran and protein or their peptides in fermented milk were important in relation to lymphocytes.

Bioactive peptides and proteins from fermented milk products have important health benefits such as stimulating effects on lymphocyte proliferation and immunoglobulin production [45]. Fermented milk such as kefir has a role as immunomodulator due to kefiran (exopolysaccharides) and putative immunomodulin protein in the kefir supernatant that has a molecular weight higher than 30 kDa [3,46]. In adaptive immunity, lymphocytes act as membrane receptors that recognize a broad range of non-self antigens and allow them to generate specific responses to remove invading pathogens and infected or tumoral cells [47].

Goat milk kefir without glucomannan supplementation in HFHF rats could decrease the PLT. This result differed with a previous study by [48] that ingestion of neither conventional yogurt nor H61-fermented milk caused platelet count significantly increased at 4 wk compared with 0 wk in both groups. The difference result between this study and a previous study may be due to differences in the type of fermented milk, animal subject and condition. Because goat milk kefir without porang glucomannan in this study able to decrease PLT number, this indicate the kefir has antithrombotic activity. In a recent study, suggest that fermented milk products (goat milk yogurt and ewe milk yogurt) have stronger antithrombotic properties as opposed to cow milk yogurt. In addition, the antithrombotic properties related to phosphatidylcholine derivatives in milk, although the mechanism is not yet known [49]. However, according to [50], fermented foods have antithrombotic activity due to either bioactive components or enzymes produced during fermentation which have the capability to lower thrombus production. The

antithrombotic properties of fermented foods is important to prevent emerging cardiovascular diseases.

Conclusion

Goat milk kefir supplemented with porang glucomannan in fermentation process could improve the health of rats fed a high-fat/high-fructose diet, through decreasing in plasma triglycerides and total cholesterol. Goat milk kefir also had an immunomodulatory effect on rats fed a high-fat/high-fructose diet, through the decreasing number of neutrophils that function in the first line of defense, and increasing the

lymphocytes which plays a role in adaptive immunity. The antithrombotic activity of goat milk kefir is an interesting subject for further study. The bioactive substances in goat milk kefir that may have antithrombotic activity should be further analyzed their mechanism explained.

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