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INFLUENCE OF DIETARY EGGURT ON SERUM CHOLESTEROL LEVEL IN RATS (*Rattus norvegicus*)

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Abstract

The experiment was conducted to determine the values of serum cholesterol. The effect of eggurt on the serum cholesterol were investigated in rats, assigned to five dietary treatments for 63 days: 1) Commercial rodent chow; 2) Commercial rodent chow plus a 10% of fat-enriched; 3) Commercial rodent chow plus a 10% of fat-enriched and non fermented milk; 4) Commercial rodent chow plus a 10% of fat-enriched and eggurt; and 5) Commercial rodent chow plus a 10% of fat-enriched and eggurt made from milk inoculated with starter culture of *Streptococcus thermophilus* and *Lactobacillus delbruechkii spp. bulgaricus*. The two-way analysis of variance was used to determine variation within and between dietary periods for serum cholesterol. The Duncan's multiple range tests for mean separation was used to determine the statistical significance within and between dietary periods of the same variables. There was no significant change in serum cholesterol in rats fed with milk fermented by 3% starter culture (Eggurt 3%) along with milk fermented by 6% starter culture (Eggurt 6%) for 63 days. The mean values for serum cholesterol concentrations was significantly decreased 13.25% and 16.06% when eggurt 3% and eggurt 6% respectively was fed on the day 63. The hypocholesterolemic effect produce by *Lactobacillus delbruchkii spp. bulgaricus* might be considered as indirect evidence of the permanence of the *Lactobacilli* in the gut.

Key words: Eggurt, Serum cholesterol, *Probiotic*

Introduction

Eggurt is one of milk-fermented product with the addition of egg albumen. Milk is combined with egg albumen to make a more suitable media for the growth of lactic acid bacteria. Microbial test show that the final products to be free of pathogens and the sensory evaluation indicate that the product would have an extended shelf life at refrigerator temperatures (Lin and Cunningham, 1984).

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Recently, yoghurt and other fermented milk products have been reported to contain some substances that lower serum cholesterol (Akalin et al.,1997). Besides that, fermented foods may reduce the serum cholesterol concentration by reducing the intestinal absorption of dietary and endogenous cholesterol (Havenaar and Huis in't Veld, 1992). In-vitro studies showed that *Lactobacillus* strain assimilates certain amounts of cholesterol from the culture medium, thus leading the authors to speculate that the organism binds cholesterol in the intestinal lumen thereby reducing its absorption into the circulation.

The effects of cholesterol-containing foods in the human diet has been the subject of many investigations in recent years, largely due to the hypothesized link between cholesterol and coronary heart disease (CHD). Elevated levels of cholesterol are considered to be a major risk factor for CHD. There is probably an increased incidence of atherosclerotic heart disease in patients with hypercholesterolemia (Akalin et al., 1997), which indicates the importance determining methods to reduce serum cholesterol. Level of serum cholesterol is affected by diet, age, sex, weight, genetics, activity, and disease (thyroid, liver, diabetes) and sex endogenous hormone (Krummel,1996).

The efforts to reduce atherosclerosis were developed by researchers, which include the use of food products and medicines that affected on hipocholesterolemic. The role of probiotic bacteria-containing foods has been the subject of consumers and producers. Probiotic bacteria was widely used to dairy product, especially a group of lactic acid bacteria.

The superiority of eggurt on lower serum cholesterol has not been substantiated. The objective of this investigation was, therefore, to study the effects of dietary eggurt on concentrations of serum cholesterol in rats.

Materials and Methods

Strains, Growth media, and Matherials used

The strains used were *Lactobacillus delbrueckii spp. bulgaricus* (FNCC 0040) and *Streptococcus thermophilus* (FNCC 0041) from microbiology laboratory, PAU, UGM. Pure culture of *Lactobacillus delbrueckii spp. bulgaricus* and *Streptococcus thermophilus* were inoculated in growth media, MRS Broth (deMan Rogosa Sharpe), Oxoid. Media have been used for the enumeration of probiotic bacteria is MRS Agar, Oxoid.

The other matherials used in experiments are UHT milk (PT. Ultra Jaya), skim milk Lactona (Nestle), 25 white male rats (UPHP, *Unit Pengembangan Hewan Percobaan*, UGM), and Kit's *reagen* for measuring of serum cholesterol.

Eggurt manufacture

For the production of eggurt, whole milk was heated at 90°C for 30 min prior to fermentation and divided into two parts. The portions were then cooled to 40°C, and milk was added with a 10% egg albumen. Egg albumen was prepared by pasteurization and blending. Milk and egg albumen mixture was inoculated with a 3% (vol/vol) and a 6% (vol/vol) starter culture of *Streptococcus thermophilus* (FNCC 0041) and *Lactobacillus delbrueckii spp. bulgaricus* (FNCC 0040) from microbiology laboratory (PAU, UGM), respectively. Each of the inoculated mixture was incubated at 40°C for 6 hours. The products were cooled to 5°C for 24 hours and then stored at freezer temperature (Stadelman, 1990). The number of lactobacilli in freshly fermented milk products was determined by established procedures Standard Plate Count (Houghtby et al., 1992) to be approximately 10^{12}cfu/ml.

Dietary treatments

Twenty five (25) white male rats (mean initial weight of 165 g) were fed with a commercial rodent chow and water for ad libitum intake for 1 week. Rats then were divided at random into five groups of five each. Group 1 received a commercial rodent chow, group 2 received a commercial rodent chow plus a 10% of fat-enriched, group 3 received a commercial rodent chow plus a 10% of fat-enriched and non fermented milk, group 4 received a commercial rodent chow plus a 10% of fat-enriched and eggurt with a 3% starter culture, and group 5 received a commercial rodent chow plus a 10% of fat-enriched and eggurt with a 6% starter culture. The rats received rodent chow diets for ad libitum intake for 63 days and received orally non fermented milk and eggurt diets every morning for 63 days. Weight gains were calculated for respective groups everyweek for 63 days.

Cholesterol test

Blood samples were collected from retroorbital venous (Taranto et al., 2000), at 21, 42, and 63 days, and placed in sterile tubes, followed by centrifugation at 3000 rpm for 20 min. The obtained serum samples were analyzed for cholesterol with Enzymatic colorimetric test, CHOD-PAP (Richmond, 1973).

Statistical analysis

The experimental design was a split plot in a randomized block design with a 5X4 factorial arrangement of treatments. Two-way analysis of variance was used to determine variation within and between dietary periods for serum cholesterol and feed intake of cholesterol diet. The Duncan's Multiple Range Test (DMRT) for mean separation was used to determine the statistical significance within and between dietary periods of the same variables. All data are reported as means (Astuti, 1980).

Results and Discussion

Weight gains

Weight gains of rat groups was fed commercial rodent chow (BR), commercial rodent chow plus a 10% of fat-enriched (BRF), commercial rodent chow plus a 10% of fat-enriched and non fermented milk (BRE 0%), commercial rodent chow plus a 10% of fat-enriched and eggurt starter 3% (BRE 3%), and commercial rodent chow plus a 10% of fat-enriched and eggurt starter 6% (BRE 6%) were increased for 63 days. The effect of diet supplementation with eggurt on the weight gain of rat is illustrated in figure 1. The figure 1 is shown that weight gains was increased when rats were fed with a commercial rodent chow plus a 10% of fat-enriched and eggurt for 63 days. It was concluded that dietary feed is easily digested and absorbed by digestible tract. There was no significant differences among treatments in dietary periods of the same variables.

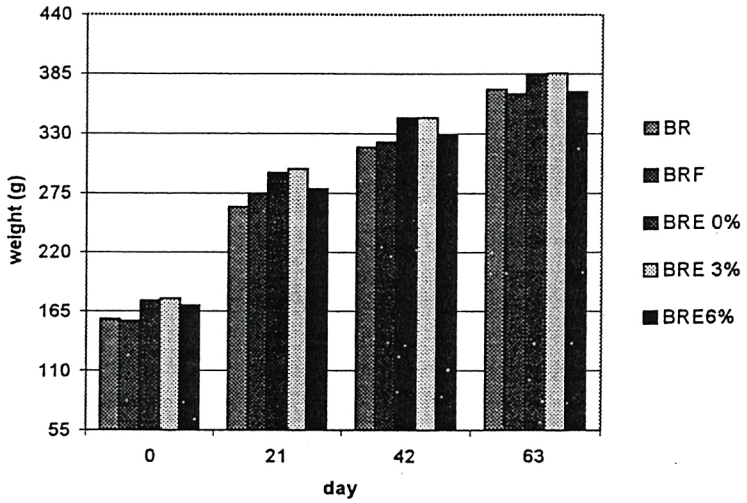

Figure 1. Effect of eggurt on weight gain of rats. BR, commercial rodent chow; BRF, commercial rodent chow plus a 10% of fat-enriched; BRE 0%, commercial rodent chow plus a 10% of fat-enriched and non fermented milk; BRE 3%, commercial rodent chow plus a 10% of fat-enriched and eggurt starter 3%; BRE 6%, commercial rodent chow plus a 10% of fat-enriched and eggurt starter 6%.

Feed intake of dietary cholesterol

Feed intake of dietary cholesterol did not differ among treatments on 21, 42, and 63 day (table 1). It was showed that increase or decrease of serum cholesterol was significantly affected by eggurt diet.

Table 1. Feed intake of dietary cholesterol

Day	Means of feed intake cholesterol diet (mg)					
	BR	BRF	BRE 0%	BRE 3%	BRE 6%	Means
7	$120.190^{a,x}$	127.835^{ab}	$132.772^{b,x}$	$131.002^{b,y}$	132.553^{b}	128.870*
14	$12.696^{a,x}$	132.230^{b}	$135.519^{bc,xy}$	$136.267^{c,y}$	133.927^{bc}	131.728**
21	121.540^{xy}	125.820	135.748^{y}	134.207^{y}	128.203	129.104^{ns}
28	128.799^{y}	132.047	137.580^{y}	128.713^{xy}	129.348	131.297^{ns}
35	138.759^{z}	127.651	129.338^{y}	129.399^{xy}	135.300	132.089^{ns}
42	127.786^{y}	126.003	120.410^{y}	120.471^{x}	126.372	124.208^{ns}
49	131.331^{yz}	122.707	112.695^{y}	121.387^{x}	124.999	122.624^{ns}
56	136.395^{z}	124.172	132.772^{y}	129.628^{y}	128.432	130.280^{ns}
63	142.135^{z}	130.215	135.748^{y}	145.653^{z}	134.614	137.673^{ns}
Means	129.737**	127.631^{ns}	130.287*	130.747**	130.416^{ns}	

^{ns} not significant (P>0.05)
^{a,b} Means within a row with no common superscript letters differ (P<0.05)
^{x,y,z} Means within a column with no common superscript letters differ (P<0.05)

Level of serum cholesterol is depend on endogenous and exogenous cholesterol, and diet (Harper et al., 1979). The exertion to decrease serum cholesterol with consider diet is an effective, such as dietary eggurt.

Eggurt is one of beneficially milk-fermented product for diet, because it contains of 1).Lactic acid which purpose as barrier pathogens bacteria in the gut and support *Bifidobacteria* growth (Mitsuoka, 1989); 2)Probiotic bacteria which keeps balance of intestines flora and decreases serum cholesterol; and 3)Egg-white (albumen) which purpose to increase permeability of intestines wall on bacteria (Romanoff and Romanoff, 1963).

Serum Cholesterol

The effect of eggurt (milk-fermented by 0%, 3%,6% starter culture) on serum cholesterol is shown in table 2.

Table 2. Effect of dietary eggurt with a 3% and with a 6% starter culture on serum cholesterol in rats (mg/dl) at 0, 21, 42, and 63 d

Dietary treatment	Cholesterol level (mg/dl)				
Group[1]	0 d	21 d	42 d	63 d	Means
Control (rodent chow)	54.65^{x}	$45.67^{a,x}$	57.78^{xy}	$70.59^{b,y}$	57.17*
Rodent chow plus fat-enriched 10%	60.85^{y}	$46.57^{a,x}$	58.89^{y}	$71.77^{b,z}$	59.52*
Non fermented milk	61.41^{y}	$43.58^{a,x}$	64.44^{y}	$72.94^{b,y}$	60.59*
Eggurt with 3% starter culture	64.23	55.52^{b}	52.22	51.06^{a}	55.76^{ns}
Eggurt with 6% starter culture	53.18	51.46^{ab}	51.11	49.41^{a}	51.29^{ns}
Means	58.86^{ns}	48.56^{*}	56.89^{ns}	63.15*	

^{ns} not significant (P>0.05)
^{a,b} Means within a column with no common superscript letters differ (P<0.05).
^{x,y,z} Means within a row with no common superscript letters differ (P<0.05).

On 63 day, level of serum cholesterol in rats were fed with eggurt 3% (BRE 3%) and eggurt 6% (BRE 6%) are significantly lower than rats were fed with a commercial rodent chow (BR), commercial rodent chow plus a 10% of fat-enriched (BRF), and non fermented-milk (BRE 0%), whereas feed intake of cholesterol diet did not differ among treatments. It was included that decreasing of serum cholesterol was affected by eggurt diet.

The mean values for serum cholesterol concentrations was significantly decreased 13.25% and 16.06% when eggurt 3% and eggurt 6% respectively was fed for 63 days, whereas level of serum cholesterol in rats were not fed with eggurt was increased 16.25% (BR), 17.99% (BRF), and 19.30% (BRE 0%) (Figure 2). This decreasing is caused by *Lactobacillus delbrueckii spp. bulgaricus* have been suppressed a new formation of LDL (Low Density Lipoprotein) with by affect activity of hydroxymethylglutaryl Co A reductase (HMGCoA reductase) (Lichtenstein and Goldin, 1993). In the gut, *probiotic* bacteria of eggurt can assimilate cholesterol become to non-absorbable *coprostanol* (Anonym, 1989), and then was excreted with feces, so absorption of cholesterol reduced in circulation.

Hyphocholesterolemic effect was caused by eggurt which probably contains 3-hydroxy-3-methylglutaric acid or *orotic* acid which barrier synthesis of cholesterol. It was suggested by Mann (1977) *cit.* Jay (1992), that dietary of yogurt was decreased serum cholesterol to human subject, because yogurt consist 3-hydroxy-3-methylglutaric acid or orotic acid.

Feeding diet of eggurt 3% and eggurt 6% did not affect on level of serum cholesterol yet on 21 day and 42 day. It is suggested by research of Akalin et al. (1997), that *Lactobacillus delbrueckii spp. bulgaricus* did not affect on level of serum cholesterol on 28 day and beginning affected on serum cholesterol on 56 day. Jay (1992), said yogurt, which contain *Lactobacillus delbrueckii spp. bulgaricus,*

Streptococcus thermophillus, and *Lactobacillus acidophilus* did not affect on serum cholesterol of rats on 21 day and 28 day. It was concluded that *probiotic* bacteria was still adapting to growth as flora intestines. No significant difference (P>0.05) was found between the treatments for BRE 3% and BRE 6% (table 2.)

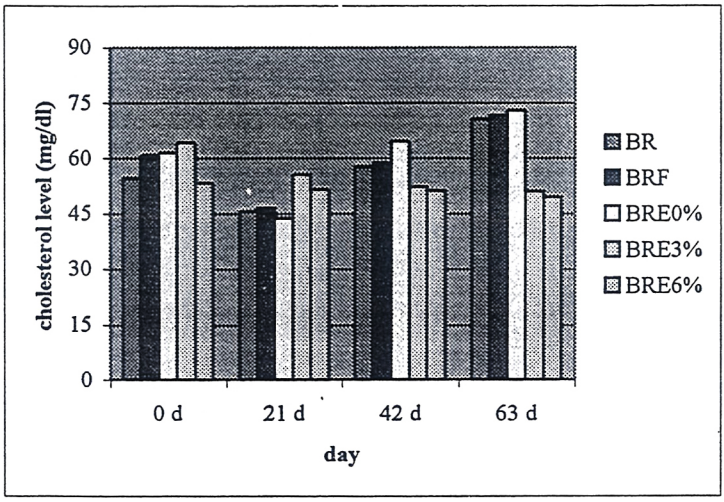

Figure 2. Effect of dietary eggurt with a 3% and with a 6% starter culture on serum cholesterol in rats (mg/dl) at 0, 21, 42, and 63 d. BR, commercial rodent chow; BRF, commercial rodent chow plus a 10% of fat-enriched; BRE 0%, commercial rodent chow plus a 10% of fat-enriched and non fermented milk; BRE 3%, commercial rodent chow plus a 10% of fat-enriched and eggurt starter 3%; BRE 6%, commercial rodent chow plus a 10% of fat-enriched and eggurt starter 6%.

Conclusions

The dietary eggurt with a 3% and 6% starter of *Lactobacillus delbrueckii spp. bulgaricus*, and *Streptococcus thermophillus* was decreased serum cholesterol 13.25% and 16.06% respectively for 63 days. It was concluded that *probiotic* bacteria in eggurt has capability to decrease serum cholesterol.

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