

## The Effect of Cocoa and Fermented Milk Mixture on Triglyceride and Blood Glucose Contents of Hyperlipidemia Rats

Lovita Adriani<sup>1\*</sup>, Nenden Indrayati<sup>2</sup> and Sjafril Darana<sup>1</sup>

<sup>1</sup>Faculty of Animal Husbandry, Padjadjaran University, Bandung, Indonesia

<sup>2</sup>Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung, Indonesia

\*Corresponding author. E-mail: lovita\_yoghurt@yahoo.co.id

### ABSTRACT

*Chocolate can undergo fortification process to be added into other food such as probiotic yoghurt. Fortification is usually regarded as the deliberate addition of one or more micronutrients to particular foods, so as to increase the intake of these micronutrient(s) in order to correct or prevent a demonstrated deficiency and provide a health benefit. The study objective was to investigate the effect of cocoa powder and fermented milk mixture to reduce triglyceride and blood glucose content on hyperlipidemia rats. Experimental design in this research was a Completely Randomized Design with five treatments, i.e. R0 = control, ration with high fat, R1 = high fat diet + 4 ml yoghurt, R2 = high fat diet + 0.35 g cocoa, R3 = high fat diet + 4 ml yoghurt + cocoa 0.23 g, R4 = high fat diet + 4 ml yoghurt + cocoa 0.35 g, R5 = high fat diet + 4 ml yoghurt + cocoa 47 g; each treatment was repeated for five times. From the statistical analysis it was indicated that the effect from the addition of cocoa and probiotic in ration, showed a significant effect ( $P < 0.05$ ) on decreased triglyceride serum level of the hyperlipidemia rats, although the content of blood glucose did not significantly different. The results showed that mixture of cocoa and probiotic in all treatments has reduced the triglyceride level until R2= 35.35%, R3= 35.66%, R4= 38.99%, R5= 38.95%, R6=40.39%, but instead, for blood glucose content there was no significance among all treatments.*

**Key words:** Hyperlipidemia rat, Probiotic, Cocoa, Triglyceride

### INTRODUCTION

Cocoa was named *Theobroma* by Linnaeus, which means 'food of the gods'. It was so called from the goodness of its seeds (Grieve, 2010). Cocoa beans are used in chocolate production. Chocolate/cocoa contains flavonoids, a type of polyphenol antioxidant. Antioxidant can reduce cancer by attacking actively oxygen compounds which are carcinogen to our body. Some researchers found that chocolate may lower blood pressure of people with hypertension. Beside that, it also can reduce LDL cholesterol level (Gloria Tsang, 2006). Cocoa contains large amounts of flavonoids–flavanols (epicatechins and catechins), anthocyanins and proanthocyanidins. The cocoa epicatechins and procyanidins make the insulin work better. It increases insulin sensitivity and forces the blood sugars faster in the cells. It makes the sugars burn faster and helps stabilize the overall blood sugars.

It is truly amazing what the correctly processed cocoa can do in a short period of time. The different chemicals - theobromine, serotonin, and give the additional energy. Cocoa also stabilizes blood sugar and makes people feel stronger more quickly.

Raw cocoa contains calcium, phosphorus, iron, thiamine (vit B1), riboflavin (vit B2), niacin, nicotinamide (vit B3), pantothenic acid (vit B5), pyridoxine (vit B6), ascorbic acid (vit C), magnesium, copper, zinc, manganese, and vitamin E.

Food fortification is usually regarded as the deliberate addition of one or more micronutrients into particular foods to increase the intake of these micronutrients in order to correct or prevent a demonstrated deficiency and provide a health benefit. Chocolate can undergo fortification process to be added into other food like probiotic yoghurt. So chocolate and probiotic yoghurt itself can

reduce LDL concentration in our body. In proving the above matter to be correct, an experiment was conducted to see whether the combination of chocolate and probiotic yoghurt can increase effect of reducing LDL concentration in rat blood. The result will hopefully benefit the community and therefore utilizing fortified cocoa in probiotic yoghurt for prevention of coronary artery disease. So if we add fortified cocoa in probiotics yoghurt, the effect may be better than probiotics yoghurt itself in decreasing LDL in serum plasma.

Triglycerides or triacylglycerols (TAG), which are esters of the trihydric alcohol glycerol, and fatty acids TAG biosynthesis mostly occur in endoplasmic reticulum. It started from fatty acids that were activated by acyl-CoA synthetase to form acetyl-CoA. Two molecule of acetyl-CoA were combined with glycerol-3-phosphate to form phosphatidate (1,2-diacylglycerol phosphate). Phosphatidate was transformed into 1,2-diacylglycerol by phosphatidate phosphohydrolase. Then the diacylglycerol will undergo esterification with acetyl-CoA and form TAG, which will be catalyzed by diacylglycerol acyltransferase. TAG are transformed into 2-monoacylglycerol by pancreatic lipase in the small intestine. TAG functions as fatty acids storage in liver and adipose tissues.

Triglycerides can be described as the main form of fats in a food item. The excess calories consumed, which are not used by the body in the form of energy, are converted into triglycerides and stored as body fat.

## MATERIALS AND METHODS

In this experiment animal samples were used which consisted of 36 adult male Wistar rats. Those rats were about 2-3 months old and weighed about 200-250 g. They got treatment for 37 days, which consisted of 2 days adaptation, 7 days of pre-condition with hypercholesterol feed, and 28 days of treatment. In this study, Completely Randomized Design was used and statistically analyzed. The 36 rats were randomly divided into 6 groups which consisted of 3 rats in each group, and repeated twice for each treatment.

At the end of the treatment, a sufficient amount of rat blood was taken by cutting the edge of its tail to analyze the lipid profile and blood glucose using the microhematocrite pipet.

### Material used in experiment

This study used the fortified cocoa (*Theobroma cacao*) powder in probiotic yoghurt as a preventive medicine to reduce the triglyceride and blood glucose level in white male rats. The cocoa powder was obtained from pure cocoa from Ceres, a commercial food factory in Bandung. Rat's standard food (pellet) and aquadest were also needed in this research. Probiotic yoghurt is taken from product of Lovita yoghurt.

The cocoa concentration of 12, 15 and 18% were used, along with a constant amount of probiotic yoghurt. According to Lovita, a person should consume about 250 ml per day. Those values were converted to be given to the rats which weighed 200 g. Based on the comparison of body weight of human and rats, the dosage for the rats was 4 ml per day. The concentration of probiotic yoghurt that was given to the rats was calculated following the research of Lovita (2005). In this study, the 36 rats were randomly divided into 6 groups which consisted of 3 rats in each group, and repeated twice for each treatment.

Normal consumption of cocoa powder for humans according to the research of Ochanomizu University, Japan, is from 13–36 g per day. Based on the research, it was decided to use 3 different concentrations, each value 13, 19.5 and 26 g. Those values were converted to be given to the rats based on the comparison of body surface areas of human and the rats:

$$\begin{aligned} \text{Rat dosage (200 g)} &= \text{Human dosage (70 kg) x body surface area comparison of rat} \\ &\quad \text{to human (conversion factor)} \\ &= 13\text{g/day} \times 0.018 = 0.23 \text{ g/day} \end{aligned}$$

$$\text{Empirical concentration} = 0.23 \text{ g} \rightarrow 0.23 \text{ ml}$$

$$= \frac{0.23}{4 \text{ ml}} \times 100 = 5.8 \%$$

With the same formula, we calculated that the empirical concentration for dosage of 19.5 g is 8.8%, and for dosage of 26 g is 11.8%. This concentration was given to the rat in the study. Feed composition followed the theory of Reeves et al. (1993), where a value of 1% cholesterol was added for hypercholesterolemic condition.

The treatments were:

- Group 1: hypercholesterol feed
- Group 2: hypercholesterol feed + probiotic yoghurt 4 ml
- Group 3: hypercholesterol feed + cocoa 0.23 g
- Group 4: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.23 g
- Group 5: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.35 g
- Group 6: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.46 g

### RESULTS AND DISCUSSION

In Table 1, the level of triglyceride in tested animal blood was reduced after consuming fortified cocoa in probiotic yoghurt. The effect seemed better than only consuming probiotics yoghurt or cocoa itself for decreasing the amount of triglyceride. All treatments showed significantly different results compared with hyperlipidemia food. R2 (yoghurt without cocoa) and R3 (cocoa without yoghurt), showed a little decrease triglyceride level, while R4, R5, and R6 seemed to be significantly different compared with R1, R2 and R3, which means that the use of yoghurt fortified or mixed with cocoa can reduce triglyceride levels than the use of cocoa or yoghurt separately. High levels of fat deposits usually also have high triglyceride amount level. Triglyceride in body comes from two different sources, from diet and from liver, which have been brought along together with the VLDL cholesterol (Yuan et al., 2007). It can be concluded that hypercholesterol feeding would result in a static blood triglyceride. In the same table, the amount of blood glucose from all treatment is not significantly differ. The cocoa's epicatechins and procyanidins make the insulin works better. It increases insulin sensitivity and forces the blood sugars faster in the cells. It makes the sugars burn faster and helps stabilize the overall blood sugars.

**Table 1.** Effect of treatment on triglyceride level and blood glucose.

Treatment	R1	R2	R3	R4	R5	R6
	.....mg/dl.....					
Triglyceride	243.25a	157.25b	156.50b	148.50c	145.25c	145.00c
Blood glucose	117.5	130.33	110.25	115.2	104	112.5

Note: The same letter on the same line show no significant difference ( $P < 0.05$ ).

### CONCLUSION

1. Consuming fortified cocoa in probiotic yoghurt proved to be better than only the probiotic yoghurt or cocoa itself in reducing the amount level of triglyceride.
2. Blood glucose level is almost same in all treatments.

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