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The 2\textsuperscript{nd} International Seminar
“Feed Safety for Healthy Food”

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Proceeding
The 2nd International Seminar
“Feed Safety for Healthy Food”

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FOREWORD

We thank the Almighty Allah, the Most Gracious and the Most Merciful that the proceedings of the 2nd International Seminar, the 8th Biannual Meeting and 3rd Congress and Workshop of AINI with the theme “Feed Safety for Healthy Food” organized by Indonesian Association of Nutrition and Feed Science, Faculty of Animal Husbandry, Universitas Padjadjaran on 6 - 7 July 2011 have been completed.

These activities were to collect variety of scientific information with the purpose to collect scientific information about feed for a healthy food, to produce a draft policy on a national feed system and to make a scientific forum for Academics, Researchers, Practitioners of animal husbandry, Health and Policy makers. Scientific papers that were presented either in oral or poster stated in the proceedings.

Thanks go to all those who have provided both moral support or material so that this seminar can be carried out and the proceeding can be issued.

Jatinangor, 6 Mei 2012

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THE ADDITION OF COCOA (*Theobroma cacao*) POWDER IN MILK FERMENTED TO REDUCE THE URIC ACID LEVEL ON HYPERLIPIDEMI RATS

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**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 to increase the intake of these micronutrients in order to correct or prevent a demonstrated deficiency and provide a health benefit.

The study object was to investigate the effect of addition cocoa powder in milk fermented milk mixture to reduce the uric acid level on hyperlipidemia rats.

In this research was used a Completely Randomized Design experimental method with five treatments, i.e. R0 = control, high fat diet, 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 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 the uric acid level on hyperlipidemi rats.

The results showed that addition cocoa into probiotic in all treatment has reduced the uric acid level, i.e. R1= 34,03%, R3= 29,9 %, R4= 29,9 %. The highest for reducing uric acid is R5= 37,11%, and the lowest is R2= 14,03%, compare to control

Keywords: hyperlipidemi rat, probiotic, cocoa, uric acid

**INTRODUCTION**

Cocoa was named *Theobroma* by Linnaeus, which means 'food of the gods'. It was so called from the goodness of its seeds (Grieve M. A., 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 R, 2006). Flavonoids in cocoa are flavanols (epicatechins and catechins), anthocyanins and proanthocyanidins. The cocoa epicatechins and procyanidins make stabilize the overall blood sugars. Consume smaller amounts of very dark chocolate (providing 30 mg of polyphenols per day) for a much
longer period of time, there is an improvement in endothelial cell relaxation, but without a reduction of blood pressure.

Probiotic yoghurt is basically yoghurt with live and active cultures. It can promote and maximize digestion of certain nutrients in the human body. Yoghurt contains such as: energy, protein, fat, carbohydrates, minerals and has a fairly complete content of vitamins are: vitamin A, B complex, B1 (thiamin), B2 (riboflavin), B6 (pyridoxine), B12 (cyanocobalamin), vitamin C, vitamin D, E, folic acid, nicotinic acid, pantothenic acid, biotin and choline (Lovita, A, 2005). Normally, the body eliminates enough uric acid in the urine or through the intestines to keep its concentration at a healthy level. Only 5% of those with hyperuricaemia develop clinical symptoms of gout. Uric acid is an end-product compound from the breakdown of purines. About two thirds of purine is generated from within the body, while one third comes from the diet (Fam 2005).

Yoghurt can help to reduce uric acid levels. Also help to reduce the risk of gout. Uric acid is a divalent acid, but the second dissociation constant is so small that at around pH 7 only the monobasic salts are formed. Uric acid is a purine synthesized by a series of reactions that also are used for synthesis for other purine such as adenine and guanine, which are component of DNA. The final step the uric acid synthesis is controlled by the enzyme xanthine oxidase, a molybdenum containing enzyme. Xanthine oxidase is an enzyme that responsible for converting purine became uric acid. The most important structure in purine biochemistry is the nucleotide consisting of a purine base, ribose or deoxyribose, and phosphoric acid, but free hypox.

Theobromine, serotonin, in cocoa 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 (Frederic W. et al, 2006). Uric acid is the final product or waste products resulting from metabolism / breakdown of purines. Uric acid is antioxidants in animals, but when amounts in blood increase or reach the saturation level will experience a crystallization. The content of uric acid on rat uric acid in, whereas in female mice at 2.92 ± 0.241 mg / dl (Taconic Technical Laboratory, 1998 in Kusmiyati, 2008).

Uric acid is a derivate of purine alkaloid compounds (xanthine). Uric acid are semisolid organic compounds consisting of carbon, oxygen, nitrogen, and hydrogen with the formula C5H4N4O3, which is the end of protein and purines metabolism.

Xanthine oxidase catalytic is an enzyme that catalyzes hypoxanthin and xanthin to uric acid, which is a purine degradation pathway, in normal tissue. xanthin oxidase is dehydrogenase. In normal tissue, xanthin oxidase is a dehydrogenase that uses NAD as an electron acceptor in the purine degradation pathway.
MATERIALS AND METHODS

In this experiment was used animal sample, which consisted of 36 adult male Wistar rats. Those rats are about 2-3 months old and weigh about 200-250 gram. They get 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, was used Completely Randomized Design. The 36 rats are randomly divided into 6 groups which consists of 3 rats in each group, and repeated twice for each treatment.

At the end of the treatment, the sufficient amount of rat blood was taken by cutting the edge of its tail to analyze the uric acid level using the microhematoctite pipet.

Material Used In Experiment

This study used the fortified cocoa \( \text{(Theobroma cacao)} \) powder in probiotic yoghurt as a preventive medicine to reduce the uric acid level in rats. The cocoa powder was obtained from pure cocoa from Ceres, a commercial food factory in Bandung. Rat's standard food is pellet and aquadest, also probiotic yoghurt is taken from product of Lovita Yoghurt Unpad.

The cocoa concentration of 12%, 15% and 18% were used, along with a constant amount of probiotic yoghurt. According to Lovita, Unpad, a person should consume about 250 ml per day. Those values are converted to be given to the rats which weigh 200 gram. Based on the comparison of body weight of human towards the rats, the dosage for the rats is 4 ml per day. The concentration of probiotic yoghurt that will be given to the rats will be calculated following the research of Lovita (2005)

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

\[
\text{Rat dosage (200 gram)} = \text{Human dosage (70 kg) \times body surface area comparison of rat to human (conversion factor)}
\]

\[
= 13g/day \times 0.018 = 0.23 \text{ g/day}
\]

\[
\text{Empirical concentration} = 0.23 \text{ g} \rightarrow 0.23 \text{ mL}
\]

\[
= \frac{0.23}{4} \text{ mL} \times 100 = 5.8 \%
\]

With the same formula, 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 formula treatments are:

- Group 1: hypercholesterol feed
- Group 2: hypercholesterol feed + probiotic yoghurt 4 ml
- Group 3: hypercholesterol feed + cocoa 0.23 gr
- Group 4: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.23 gr
- Group 5: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.35 gr
- Group 6: hypercholesterol feed + probiotic yoghurt 4 ml + cocoa 0.46 gr
RESULTS AND DISCUSSIONS

Effect Treatments on Uric Acid Level

Table 1. Effect of treatment on uric acid level (mg/dl)

<table>
<thead>
<tr>
<th></th>
<th>R0</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>ab</td>
<td>n</td>
<td>ab</td>
<td>ab</td>
<td>b</td>
</tr>
<tr>
<td>4.85</td>
<td>3.2</td>
<td>4.17</td>
<td>3.4</td>
<td>3.4</td>
<td>3.05</td>
<td></td>
</tr>
</tbody>
</table>

R0 = high fat diet
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

In Table 1 and Figure 1, showed that the level of uric acid in tested animal blood was reduced after consuming mixed or fortified cocoa in probiotic yoghurt. The effect almost the same for consuming only cocoa (R2) and R0 (control). R1 (consuming only probiotic), R3 (probiotic with little dose cocoa) and R4 (probiotic with medium dose cocoa) seemed better than R0 and R2, for decreasing uric acid level. Consume only yoghurt probiotic is better than consume only cocoa for reducing uric acid level. R5 showed significantly different compared to all treatments, which means that the use of cocoa mixed with yoghurt can reduce the uric acid levels than using cocoa or yoghurt separately. The main contributors for reducing uric acid is antioksidan. The cocoa’s epicatechins and procyandins make compounds inhibit the activity of the reaction of xanthine oxidase and superoxide so that the levels of uric acid reduce (Pitman JR, and Bross MHL, 1999) Polyphenol compounds is also a diuretic, so it will dissolve and uric acid in urine wasted, like work allopurinol in lowering uric acid level using an inhibitory pathway enzyme xanthin oxidase.
CONCLUSION

1. Consuming mixed cocoa in probiotic yoghurt proved to be better than only the probiotic yoghurt or cocoa itself in reducing the level of uric acid.
2. Consuming only cocoa is almost the same with control, only reduce 14.03% uric acid level.
3. R1, R3, R4 mixed cocoa in yoghurt can reduce uric acid until 34.03%, 29.9%, 29.9% respectively.
4. R5 is the highest for reducing uric acid level until 37.11 % compare to control.

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