นิพนธ์ต้นฉบับ

ผลของน้ำมันมะพร้าวและน้ำมันรำข้าวต่อระดับไขมันในเลือดหนูทดลอง

วันเพ็ญ มีสมญา[1,*] ศุภมาส โชติเมธีภิรมย์[2] เยาวดี คุปตะพันธ์[1] ดวงจันทร์ เฮงสวัสดิ์[1]
ผ่องศรี จิตตนูนท์[1] สุคนธ์ชื่น ศรีงาม[3]

[1]ฝ่ายโภชนาการและสุขภาพ สถาบันค้นคว้าและพัฒนาผลิตภัณฑ์อาหาร [2]ภาควิชาสรีรวิทยา คณะสัตว์แพทย์ศาสตร์
[3]ภาควิชาวิทยาศาสตร์และเทคโนโลยีการอาหาร คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์
บางเขน 50 ถนนพหลโยธิน จตุจักร กรุงเทพฯ 10900

บทคัดย่อ

การทดลองครั้งนี้เป็นการเปรียบเทียบผลของการกินน้ำมันมะพร้าวและน้ำมันรำข้าวต่อระดับของโคเลสเตอรอลรวม ไตรกลีเซอไรด์ เอชดีแอล-โคเลสเตอรอล และแอลดีแอล-โคเลสเตอรอล ในหนูทดลองเพศผู้พันธุ์ Sprague-Dawley จำนวน 40 ตัว ที่เลี้ยงด้วยอาหารทดลองที่มีปริมาณของน้ำมันพืชต่างกัน 4 ระดับ โดยสูตรที่ 1 ประกอบด้วย น้ำมันรำข้าวอย่างเดียว สูตรที่ 2 น้ำมันรำข้าว ร้อยละ 50 ผสมน้ำมันมะพร้าว ร้อยละ 50 สูตรที่ 3 น้ำมันมะพร้าวอย่างเดียว และสูตรที่ 4 น้ำมันมะพร้าว ร้อยละ 50 ทั้งนี้อาหารแต่ละสูตรประกอบด้วยแป้งข้าวโพด ร้อยละ 35 เคซีน ร้อยละ 10 น้ำมันพืช ร้อยละ 8 (ยกเว้นสูตรที่ 4 – น้ำมันพืช ร้อยละ 4) น้ำ ร้อยละ 5 เกลือแร่ ร้อยละ 5 วิตามิน ร้อยละ 1 เซลลูโลส ร้อยละ 1 และน้ำตาล ร้อยละ 35 หลังจากเลี้ยงหนู 4 กลุ่ม กลุ่มละ 10 ตัว เป็นเวลา 4 สัปดาห์ ด้วยอาหารทดลอง 4 ชนิด ๆ ละกลุ่ม เก็บเลือดและวิเคราะห์ปริมาณโคเลสเตอรอลทั้งหมด ไตรกลีเซอไรด์ เอชดีแอล-โคเลสเตอรอล และแอลดีแอล-โคเลสเตอรอล พบว่าค่าเฉลี่ยของไตรกลีเซอไรด์ของหนูที่กินอาหารผสมน้ำมันมะพร้าวร้อยละ 50 (0.89 ± 0.12 mmol/L) ต่ำกว่าหนูที่กินอาหารผสมน้ำมันรำข้าวอย่างเดียวนาน 4 สัปดาห์ (1.13 ± 0.33 mmol/L) อย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ส่วนโคเลสเตอรอลรวมและเอชดีแอล-โคเลสเตอรอลในหนูที่เลี้ยงด้วยน้ำมันมะพร้าวอย่างเดียว (TC; 2.60 ± 0.22 mmol/L, HDL-C; 2.10 ± 0.16 mmol/L) หรือน้ำมันมะพร้าว ร้อยละ 50 (TC; 2.60 ± 0.20 mmol/L, HDL-c; 2.12 ± 0.16, mmol/L) ลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับหนูที่เลี้ยงด้วยน้ำมันรำข้าวอย่างเดียว (TC; 3.21 ± 0.26 mmol/L, HDL-c; 2.48 ± 0.22 mmol/L) หรือน้ำมันรำข้าว ร้อยละ 50 ผสมน้ำมันมะพร้าว ร้อยละ 50 (TC; 3.30 ± 0.19 mmol/L, HDL-c; 2.56 ± 0.15 mmol/L) ($p < 0.05$) อย่างไรก็ตาม อาหารทั้ง 4 สูตร มีผลต่อระดับแอลดีแอล-โคเลสเตอรอลไม่แตกต่างกัน จากการทดลองนี้จะเห็นได้ว่าระดับโคเลสเตอรอลรวมในหนูทดลองที่ได้รับน้ำมันมะพร้าวต่ำกว่าหนูทดลองที่ได้รับน้ำมันรำข้าวไม่ได้มีผลจากระดับของแอลดีแอล-โคเลสเตอรอลลดลงแต่เป็นเพราะการลดลงของเอชดีแอล-โคเลสเตอรอล

***คำสำคัญ* :** น้ำมันมะพร้าว น้ำมันรำข้าว โคเลสเตอรอล ไตรกลีเซอไรด์ หนูทดลอง

*Corresponding author. E-mail: ifrwpm@ku.ac.th, Tel.: 02-942-8629-35 ext 901, Fax: 02-940-6455

วันเพ็ญ มีสมญา และคนอื่นๆ. "ผลของน้ำมันมะพร้าวและน้ำมันรำข้าวต่อระดับไขมันในเลือดหนูทดลอง" *วารสารโภชนาการ* 44, 2 (เม.ย.-มิ.ย. 2552) 58-65

Introduction

Coconut oil is important edible oil traded in the international market, accounting for around 6.4% of the total traded edible oil. In the last five years, the volume of coconut oil traded has tended to increase by 4% annually. The volume of 1.29 million tons traded in 1991 was increase to about 1.58 million tons in 1996. Two main exporters of coconut oil were Philippines and Indonesia whose export shares in 1996 were 65.6% and 20.8%, respectively.[1]

Coconut oil is liquid at temperature of 76°F or above, and solid at temperature under 76 °F. It contains medium chain fatty acids (MCFAs) such as caprylic acid (C10:0), lauric acid (C12:0), and myristic acid (C14:0). Lauric acid presenting approximately 40% in coconut oil has the greater anti-viral activity than the other two fatty acids. Lauric acid is disease fighting and present in breast milk. The body can converts lauric acid to a fatty acid derivative called monolaurin. Monolaurin is a substance protecting infants from viral, bacterial or protozoal infections. It was reported that people living in tropical climates and consuming a diet high in coconut oil had less heart disease, cancer, and colon problems than the unsaturated fat esters are.[2]

Rice bran oil, unlike most vegetable oils, contains a relatively high proportion of non-fatty acid components or nonsaponifiable lipids. Crude rice bran oil and refined rice bran oil may contain nonsaponifiable lipids up to 5% and 1.5-2.6%, respectively. In contrast, most other refined vegetable oils contain only 0.3-0.9% of nonsaponifiable lipids. The nonsaponifiables of rice bran oil are composed of 43% sterols, 28% triterpene alcohols, 10% 4-methyl sterols, and 19% polar components. Approximately 20% of the nonsaponifiable of crude rice bran oil are in a form of oryzanol originally found in rice bran oil.[3] Oryzanol is a mixture of esters of ferulic acid (4-hydroxy-3-methoxycinnamic acid) with sterols (16% campesterol, and 7% β-sitosterol), and triterpene alcohols (30% cycloartenol, 23% 24-methylene–cycloartanol, and 22% cyclobranol).

More recently, there has been a renewed interest in the nontriglyceride components of dietary oils, especially following the discovery of the hypocholesterolemic effect of rice bran and rice bran oil. Rice bran oil has greater amounts of oryzanol and tocotrienols than most highly refined vegetable oils have. The hypocholesterolemic effect of rice bran oil can be reproduced by feeding the extracted unsaponifiable lipids alone to animals. Elevations in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) increase the risk of atherosclerosis and coronary heart disease. Numerous studies had demonstrated that edible oils containing particular saturated fatty acids (SFA) raised serum TC, and in particular, LDL-c. Also those enriched in unsaturated fatty acids by replacing SFAs could lower LDL-c.[4]

Objective

A purpose of this study was to investigate and compared the effect of coconut oil with rice bran oil on serum lipid profiles of rats after ingestion for 4 weeks

Materials and methods

Animals

Forty of three-week-old male Sprague-Dawley rats with initial weight of 50-60 g were used in this study. The differences of mean body weight within group were not more than 10 g and between group were not more than 5 g. Rats were individually caged and maintained in a controlled environment at 20-22^{o}C, 60% relative humidity, and a 12–h light–dark cycle. Animals were randomly divided into four groups. Each group consisted of ten animals. They were provided with experimental diets and water *ad libitum* for a 28-day feeding period. Food consumption was measured daily and body weights of animals were checked weekly. The experimental protocol was developed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand.

Diets

Four experimental diets were prepared from coconut oil (CNO) and rice bran oil (RBO) determined by following the AOAC method.[5] Four formulas were prepared as follows: Diet 1 – 100% RBO, Diet 2 - 50% RBO + 50% CNO, Diet 3 – 100% CNO, and Diet 4 – 50% CNO. These diets were composed of 10% protein, 8% vegetable oil (except Diet 4, 4% vegetable oil), 5% mineral mixture, 1% vitamin mixture, 1% cellulose, 5% moisture, 35% sucrose, and 35% corn starch. The experimental diets were isocaloric except the Diet 4 whose energy was lower than the rest of the Diets. The purpose of using 50% CNO in Diet 4 was to study the different levels of CNO on the changes of TG and TC levels compared to the rest of the diets. (Table 1)

Sample collection

Blood was collect only once at the termination of the experiment. After 28 day feeding, animals were fast for 16 hours before anaesthetized with diethyl ether. Blood was drawn using cardiac puncture, put into a test tube, and allowed to clot at room temperature for 30 minute. The clotted blood was centrifuged at 3,000 rpm for 10 minutes to obtain serum. Serum lipid profiles including TC, TG, LDL-c, and HDL-c were analyzed using enzymatic colorimetric procedures.

Statistical analysis

Data were analyzed and expressed as mean $\pm$ SD. Values of serum lipid profiles were compared by using ANOVA. The differences in mean among the Diets were determined by using Duncan's New Multiple Range test of SPSS program. The p-values were less than 0.05 was considered as significant.

Table 1 Compositions of raw materials in four experimental diets (g/10 kg)

Ingredients	Diet 1 (100% RBO)	Diet 2 (50% RBO + 50% CNO)	Diet 3 (100% CNO)	Diet 4 (50% CNO)
Energy (kilocalorie)	38,252	38,252	38,252	34,652
Casein	1,230	1,230	1,230	1,230
Rice bran oil	800	400	-	-
Coconut oil	-	400	800	400
Mineral	500	500	500	500
Vitamin	100	100	100	100
Cellulose	100	100	100	100
Sugar	3,635	3,635	3,635	3,835
Corn starch	3,635	3,635	3,635	3,835

Results and discussion

Rats in this experiment could be used as animal models since they had shown similar characteristic of lipoproteins and cholesterol metabolism as compared with humans[6]. Lipid-containing lesions had been induced in the rat on high-fat diets. Strain of rats showing hypo- and hypercholesterolemia were developed as an aid in studying the possible genetic mechanisms in etiology[7].

Diet intake and body weight

At the beginning of experiment, initial body weight (IBW) of rats in each group was no significant difference (Table 2). After feeding with four experimental diets composed of different levels of RBO and CNO for 4 weeks, the means of final body weight (FBW) and feed intake (FI) of rats fed with Diet 1 were significantly lower than those of rats fed with Diet 2 and Diet 4. It showed that the rats preferred consuming 50% RBO + 50% CNO diet or 50% CNO diet to 100% RBO diet.

It was obvious that even though FI of rats fed with Diet 4 were significantly higher than those fed with the rest of the experimental diets, its energy intake (EI) was not significantly different from the rest of them. It was possible that Diet 4 provided calorie less than the rest of the diets. However, the feed efficiency ratio of rats fed with 50% RBO + 50% CNO diet was significantly higher than those fed with 100% RBO diet or 50% CNO diet.

Serum triglyceride

Table 3 showed that the TG-lowering effects were significantly higher in rats fed with 100% CNO diet (0.94 ± 0.17 mmol/L) or 50% CNO diet (0.89 ± 0.12 mmol/L) than those fed with the 100% RBO diet (1.13 ± 0.33 mmol/L)($p < 0.05$). It indicated that CNO alone had greater reduction serum TG than RBO alone did after ingestion for 4 weeks. However, the serum TG level of rats fed with Diet 2 containing combination of 50% CNO and 50% RBO (1.04 ± 0.16 mmol/L) was no significant difference compared with those of Diet 1 (1.13 ± 0.33), Diet 3 (0.99 ± 0.17 mmol/L), or Diet 4 (0.89 ± 0.12 mmol/L) containing only one kind of oils in different levels (Table 3). Based on the previous study, serum TG and TC levels in fourteen male Sprague-Dawley rats at eight months of age raised in the same standard conditions and fed with the normal standard control diet (composed of 24% corn meal, 20% fish meal, 12% soybean extract, 15% wheat bran, 20% rice flour, 3% mineral mixture, 2% vitamin mixture, 2% sugar, and 2% vegetable oil) were 1.29 mmol/L and 4.05 mmol/L, respectively[8]. The means of serum TG levels in all experiment groups in this study were lower than that of rats fed with standard diet. If considering energy of the four experimental diets, Diet 4 provided only 34,652 kilocalories while the rest of experimental diets contained 38,252 kilocalories. A half of fat content in Diet 4 was removed and the rest of edible oil was provided by coconut oil. The result indicated that reducing calories by 50% and using coconut oil as a source of edible oil could lower blood triglyceride level than maintaining energy supply but adjusting a ratio of rice bran oil to coconut oil. (Table 3)

Table 2 Initial body weight, final body weight, feed intake, feed efficiency ratio and energy intake in rat fed with four experimental diets for 4 weeks[1]

Diets[3]	IBW[4] (g/rat)	FBW[4] (g/rat)	FI[4] (g/rat/4 wks)	FER[4]	EI[4] (Kcal/rat/4 wks)
1	50.70 ± 4.48[a]	145.12 ± 15.36[a]	327.85 ± 38.00[a]	0.28 ± 0.03[a]	1254.09 ± 145.36[a]
2	50.62 ± 4.41[a]	164.39 ± 11.13[b]	357.59 ± 20.18[bc]	0.31 ± 0.01[b]	1367.88 ± 77.22[b]
3	51.69 ± 4.29[a]	154.59 ± 14.17[ab]	338.76 ± 32.24[ab]	0.30 ± 0.01[ab]	1295.85 ± 123.32[ab]
4	51.76 ± 4.94[a]	159.41 ± 6.43[b]	372.67 ± 21.49[c]	0.28 ± 0.01[a]	1291.39 ± 74.48[ab]

[1]mean ± SD, N = 10 *[2]Values in a column with different superscripts were significantly difference at $p < 0.05$.*

[3]Diet 1 = Diet with 100% RBO, Diet 2 = Diet with 50% RBO + 50% CNO, Diet 3 = Diet with 100% CNO, Diet 4 = Diet with 50% CNO

[4]IBW = initial body weight of rat at beginning of experiment, FBW = Final body weight of rat at 4 weeks of experiment, FI = Feed intake of rat in 4 weeks, EI = Energy intake of rat in 4 weeks, FER = Feed efficiency ratio = body weight gain / feed intake

Table 3 Serum lipid levels in rats fed with diets containing RBO or CNO for four weeks[1]

Experimental diets	TG[2] (mmol/L)	TC[2] (mmol/L)	LDL–c[2] (mmol/L)	HDL-c[2] (mmol/L)
Diet 1 100% RBO	1.13 ± 0.33[b]	3.21 ± 0.26[b]	0.24 ± 0.08[a]	2.48 ± 0.22[b]
Diet 2 50% RBO + 50% CNO	1.04 ± 0.16[a b]	3.30 ± 0.19[b]	0.23 ± 0.05[a]	2.56 ± 0.15[b]
Diet 3 100% CNO	0.94 ± 0.17[a]	2.60 ± 0.22[a]	0.19 ± 0.04[a]	2.10 ± 0.16[a]
Diet 4 50% CNO	0.89 ± 0.12[a]	2.60 ± 0.20[a]	0.20 ± 0.07[a]	2.12 ± 0.16[a]

[1]*mean ± SD, N = 10* [2]*Values in a column with different superscripts were significantly difference at p < 0.05.*

Serum total cholesterol, LDL-c, and HDL-c

Elevations in serum TC and LDL-c increase the risk of atherosclerosis and coronary heart disease[9]. Numerous studies have demonstrated that oils containing particular SFAs raise serum TC, and especially LDL-c while those enriched in unsaturated fatty acids lower LDL-c[10,11],

When considering serum cholesterol, it was found that mean serum TC and HDL–c levels in rats fed with Diet 1 and Diet 2 (LDL-c; 3.21 ± 0.26 mmol/L vs. 3.30 ± 0.19 mmol/L and HDL-c; 2.48 ± 0.22 vs. 2.56 ± 0.15 mmol/L, respectively) or Diet 3 and Diet 4 (LDL-c; 2.60 ± 0.22 mmol/L vs. 2.60 ± 0.20 mmol/L and HDL-c; 2.10 ± 0.16 mmol/L vs. 2.12 ± 0.16, mmol/L, respectively) were not significantly different (*p* > 0.05). However, feeding rats with both levels of CNO could significantly lower serum TC and HDL–c levels than feeding them with 100% RBO or a mixture of 50% RBO and 50% CNO *(p<0.05).* However, there were no significant differences among serum LDL-c levels of all experimental groups. The mean values of serum LDL-c were 0.24 ± 0.08 mmol/L in Diet 1, 0.23 ± 0.05 mmol/L in Diet 2, 0.19 ± 0.04 mmol/L in Diet 3, and 0.20 ± 0.07 in Diet 4. This finding indicated that the cholesterol-lowering effect of CNO involved lowering serum HDL-c instead of LDL-c. (Table 3)

Recently, Ausman LM et al has reported that both plasma TC and LDL-c were significantly reduced in rats fed with physically refined RBO but in not rats fed with canola oil relative to coconut oil. However, plasma TG levels in all groups were not significantly different[4]. It has demonstrated that CNO rich in SFAs in forms of MCTs had a hypo-cholesterolemic effect in rats rather than RBO had. Ausman LM used physically refined RBO while our study used rice bran oil. Physically RBO used in Ausman's study contained higher content of unsaponifiables due to eliminating the normally used alkaline extraction step in the oil refining process[12,13]. Unsaponifiables were composed of plant sterols and oryzanol. It has been established in both humans and experimental animals feeding with dietary plant sterols in pharmacologic amounts could decrease serum cholesterol levels[14-16] under conditions in which the dietary fatty acid pattern was kept constant. Both plant sterols and triterpene alcohols could inhibit cholesterol absorption[17-21]. It was found that the uptake of radiolabeled cholesterol mixed in plant sterols was inhibited in intestine, and the degree of inhibition increased as the ratio of dietary plant sterol: cholesterol increased.

In this present, it is known that if the level of serum cholesterol is lowered, the incidence of new events of coronary heart disease will be reduced[9]. It obviously showed in this study that CNO had greater reduction of serum total cholesterol than RBO had. However, serum LDL-c levels had no significant difference among rats fed with either RBO or CNO for 4 weeks. When considering HDL-c or good cholesterol, the results demonstrated that serum HDL-c levels in rats fed with CNO were lower than those of RBO. The TC-lowering effect of CNO was related to the HDL-c lowering effect instead of the LDL-c lowering effect. Therefore the effect of CNO on lowering serum HDL-c needs to be considered if intending to consume coconut oil as a main source of fat in daily diet.

Conclusions

Serum TG, TC and HDL-c levels of rats fed with coconut oil were lower than those of rats fed with RBO while no significant difference among their serum LDL-c levels after ingestion the experimental diets for four weeks. However, serum total cholesterol depletion in rats fed CO may be related to the HDL-c lowering effect instead of the LDL-c lowering effect. Therefore, coconut oil should not be consumed as a main source of fat in daily diet,

Acknowledgment

The authors are deeply grateful to Public Private Technology Development and Transfer Center, Kasetsart University, Bangkok, Thailand, for giving us the valuable opportunity, suggestion and the financial support for this research.

References

1 Arifin S, Susila WR, Luntungan H, Goenadi DH. Production and development of oil palm and coconut plantations in the major producing countries. In: Leonard EC, Perkins EG, Cahn A, editors. Proceedings of the world conference on palm and coconut oils for the 21st century. Champaign, Illinois; 1999. p16-23.

2. Lee L. Coconut oil: Why it is good for you. Reprinted 2001 [cited 2008, April 17] Available from: http://www.coconutoil.com// litalee.htm

3. Rong N, Ausman LM, Nicolosi RJ. Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters. Lipid. 1997;32(3): 303-9.

4. Ausman LM, Rong N, Nicolosi RJ. Hypo-cholesterolemic effect of physically refined rice bran oil: studies of cholesterol metabolism and early atherosclerosis in hypercholesterolemic hamsters. J Nutr Biochem. 2005;16(9):521-9.

5. Official methods of analysis (AOAC). 16th ed. Association of Official Analysis Chemists, Gaithersburg: Maryland; 1998.
6. Spady Dietschy JM. Rates of cholesterol synthesis and low-density lipoprotein uptake in the adrenal glands of the rat, hamster and rabbit *in vivo*. Biochem Biophys Acta. 1985; 836:167-75.
7. Waddell CA, Desai ID. The use of laboratory animals in nutrition research. World review of nutrition and dietetics. 1981;36:206-22.
8. Mesomya W, Hengsawadi D, Cuptapun Y, Jittanoonta P, Na Thalang V. Effect of age on serum cholesterol and triglyceride levels in the experimental rats. Kasetsart J. (Nat. Sci.). 2001;35(2):144-8.
9. The Expert Panel. Report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults; NHLB1. Arch Intern Med. 1988; 148(1):36-69.
10. Mattson FH, Grundy SM. Prediction of serum-cholesterol responses of man to changes in fats in the diet. J Lipid Res. 1985;26(2):194-202.
11. Dreon DM, Vranizan KM, Krauss RM, Austin MA, Wood PD. The effects of polyunsaturated fat vs monounsaturated fat on plasma lipoprotein. JAMA. 1990;263(18):2462-6.
12. Nicolosi RJ, Rogers EJ, Ausman LM, Orthoefer FT. Rice bran oil and its health benefits. In: Marshall W, Wadsworth J, editors. Rice Science Technology. New York: Marce Dekker Ins.; 1991. p412-37.
13. de Deckere EAM, Korver O. Minor constituents of rice bran as functional foods. Nutr Rev. 1996;54:s120-6.
14. Peterson DW. Effect of soybean sterols in the diet on plasma and liver cholesterol in chicks. Proc Soc Exp Biol Med. 1951;78(1):143-7.
15. Pollak OJ. Reduction of blood cholesterol in man. Circulation. 1953;7(5):702-6.
16. Beveridge JMR, Connell WF, Mayer GA, Haust HL. Plant sterols, degree of un-saturation, and hypocholesterolemic action of certain fats. Can J Biochem. 1958;36(9):895-911.
17. Subbiah MTR. Dietary plant sterols: current status in human and animal sterol metabolism. Am J Clin Nutr. 1973;26(2):219-25.
18. Kudchodkar BJ, Horlick L, Sodhi HS. Effects of plant sterols on cholesterol metabolism in man. Atherosclerosis. 1976; 23(2):239-48.
19. Chiu GC. Mode of action of cholesterol-lowering agents. Arch Intern Med. 1961; 108:717-32.
20. Kiribuchi MK, Miura S. Tokuda S, Kaneda T. Hypocholesterolemic effect of triterpene alcohols with soysterol on plasma cholesterol in rats. J Nutr Sci Vitaminol. 1983;29(1):35-43.
21. Ikeda I, Nakashima-Yoshida K, Sugano M. Effects of cycloartenol on absorption and serum levels of cholesterol in rats. J Nutr Sci Vitaminol. 1985;31(3):375-84.