Tu-Chung Leaf Meal Supplementation Reduced an Increase in Lipid Accumulation of Chickens Stimulated by Dietary Cholesterol

U. Santoso[1], S. Ohtani[2] and K. Tanaka*
Laboratory of Animal Nutrition, Division of Bioresources and Bioproduction, Graduate School of Agriculture
Hokkaido University, Sapporo 060-0809, Japan

ABSTRACT : The effect of tu-chung (*Eucommia ulmoides*, Oliver) leaf meal on reducing lipid accumulation in chickens fed 1% cholesterol containing diet was studied. Forty male White Leghorn chickens aged 56 days were weighed and divided into four groups of ten chickens, and fed diets with or without 1% dietary cholesterol which were supplemented with 0 and 5% tu-chung. Tu-chung supplementation to the diet without cholesterol increased acetyl-CoA carboxylase ($p<0.01$) but decreased 3-hydroxy-3-methylglutaryl-CoA reductase activities ($p<0.01$) with no effect on fatty acid synthetase activities. However, its supplementation to the diet with cholesterol had no effect on these three enzyme activities as compared with the cholesterol containing diet without tu-chung. Tu-chung supplementation to the diet without cholesterol increased hepatic triglyceride ($p<0.01$), whereas its supplementation to the diet with cholesterol decreased it ($p<0.01$). Tu-chung supplementation to the diet with cholesterol decreased plasma cholesterol ester, free cholesterol, phospholipids ($p<0.05$) and triglyceride ($p<0.01$) as compared with the cholesterol containing diet without tu-chung. Supplementation of tu-chung to the diet without cholesterol decreased plasma free cholesterol ($p<0.05$). It is concluded that tu-chung leaf meal reduced an increase in lipid accumulation in chickens stimulated by 1% cholesterol feeding. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 12 : 1758-1763*)

Key Words : Tu-Chung Leaf Meal, Dietary Cholesterol, Lipid Accumulation, Chickens

INTRODUCTION

Experimental, clinical and epidemiological evidences have established a clear relationship between atherosclerosis and elevated levels of serum cholesterol (Khachadurian, 1990). Recent studies also indicate that dietary modifications and pharmacological agents that reduce serum cholesterol are effective in slowing the process of atherosclerosis. Obesity or fatty liver due to the excess intake of lipid is also a serious problem in animal production. Therefore, that disease should be prevented in order to optimize the health of animal.

Tu-chung (*Eucommia ulmoides*, Oliver) is one of the medical herbs in China that has been used to strengthen the intestinal organs and heart, to provide vigor of spirit, to remove fatigue, to prevent aging, and to strengthen bone and muscles (Chen, 1934 cited by Yokogoshi et al., 1991). However, its precise metabolic effects are not clear. Recent studies showed that tu-chung decreased lipid accumulation in the liver and adipose tissue of rats (Ma et al., 1987). Furthermore, Ohashi et al. (1991) found that in rats, supplementing a high-cholesterol diet with tu-chung leaf meal lowered serum cholesterol. In laying hens at a late production stage, tu-chung leaf meal supplementation, however, increased abdominal fat weight with no effect on plasma or egg yolk cholesterol (Muramatsu et al., 1993). Growing chickens and laying hens might have a different response from dietary treatments. There is little information, if any, on the advantages of tu-chung leaf meal on reducing lipid accumulation in growing chickens. Our preliminary experiment showed that supplementation of tu-chung at 5% was effective in lowering 3-hydroxy-3-methyl glutaryl-CoA reductase activity and plasma cholesterol in growing chickens fed a diet without cholesterol (Tanaka et al., unpublished results). However, it is unknown whether tu-chung supplementation has a beneficial effect with diets supplemented or not with cholesterol. Therefore, the present study was conducted to evaluate tu-chung effects on lipid metabolism in growing chickens fed diets with or without cholesterol. The present study hypothesized that tu-chung leaf meal may inhibit lipid accretion in the liver and plasma caused by dietary cholesterol without loss of general performance characteristics.

MATERIALS AND METHODS

Fourty male White Leghorn chickens aged 56 days were weighed and leg-banded. They were divided into four groups, and fed diets with or without 1% dietary cholesterol which were supplemented with 0 and 5% tu-chung. The chickens were raised to 77 days of age in individual cages in an air-conditioned room

* Address reprint request to K. Tanaka. Tel: +81-11-706-2476, Fax: +81-11-706-2476, E-mail: ketanaka@anim.agr.hokudai.ac.jp.
[1] Department of Animal Science, Faculty of Agriculture, Bengkulu University, Bengkulu, Indonesia.
[2] Present address: Department of Animal Science and Technology, Faculty of Agriculture, Gifu University, Gifu, Japan.
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(temperature 22±2℃ with humidity 60-70%) with the light on from 08:00 to 20:00. Our previous results showed that 1% inclusion of cholesterol in the diet was effective in increasing cholesterol contents in the liver, plasma and intestine of chickens (Youn et al., 1993). Therefore, this level was used in the present study. Body weight was individually measured weekly, and feed intake was calculated daily. Feed and water were made available at all times. All chickens were fed a commercial diet (moisture 10%, crude protein 18%, fat 5.5%, crude fiber 4%, ash 7%, NFE 55,5%, Ca 0.8%, P 0.5% and ME 3,200 kcal/kg). Commercial tu-chung leaf meal was obtained from Kanzaki Company, Ltd; Takamatsu, Japan; the chemical composition is presented in table 1.

At the end of the experiment period (77 days of age), eight chickens of each group were killed, and livers were removed and weighed. One portion of the liver was placed in ice-cold saline to measure the activities of lipogenic and cholesterologenic enzyme activities, and another portion was frozen and stored at -30℃ until analysis of various lipid fractions. Blood was also taken from a wing vein with a heparinized syringe and then centrifuged at 2,500 rpm for 10 minutes. Plasma obtained was stored and frozen at -30℃ until analysis of lipid fraction concentration. The lipid fractions were separated by thin-layer chromatography on silica gel chromarod using hexanediethyl-ether-formic acid (60:10:1) and hexane-benzene (1:1) as developing solvent and quantified by IATROSCAN TH-10 TLC/FID Analyzer (Iatron Laboratories, Inc., Tokyo, Japan).

Liver homogenates for enzyme activity were obtained by the method previously described (Santoso et al., 1993). Acetyl-CoA carboxylase (ACC) activity was assayed by the $H^{14}CO_3$ - fixation method (Qureshi et al., 1980). Fatty acid synthetase (FAS) activity was assayed by the 1-^{14}C-acetyl-CoA incorporation method (Hsu et al., 1965). 3-hydroxy-3-methylglutaryl-CoA reductase activity was assayed by the method of Shefer et al. (1973). The protein content of the solution used for enzyme assay was determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. ACC and FAS activities were expressed as nanomole of substrate converted to product per minute per milligram of protein at 37℃. 3-hydroxy-3-methylglutaryl-CoA reductase activity was expressed as picomole of substrate converted to product per minute per mg protein at 38℃. Samples were analyzed in triplicate.

All data were statistically analyzed using analysis of variance (Shinjo, 1990). Duncan's Multiple Range Test determined significant different between treatments in the same diet.

Table 1. Tu-chung leaf meal composition (in 100 g)

Nutrient	
Moisture	2.2 g
Protein	14.3 g
Fat	6.7 g
Tannin	5.64 g
P	227 mg
Ca	1.49 mg
Na	1.50 mg
K	1.5 g
Mg	281 mg
Zn	19.0 ppm
Caffeine	<0.001 g
Fiber	8.9 g
NFE	57.3 g
Fe	28.3 mg
Pectin	4.18 g
Ash	10.6 g
ME	299.6 kcal

RESULTS

Dietary supplementation of tu-chung leaf meal had no effect on body weight gain, body weight and liver weight, feed intake and feed conversion ratio ($p<0.05$) (table 2). Feed conversion ratio tended to be higher in chickens fed tu-chung-containing diet, whereas body weight gain tended to be lower. The cholesterol-containing diet had no effects on the above variables except for liver weight which was greater ($p<0.05$) than that with the non-cholesterol diet.

Greater ACC ($p<0.01$) but less 3-hydroxy-3-methyl-glutaryl-CoA reductase activity ($p<0.01$) was observed in chickens fed the non-cholesterol diet with tu-chung than in those fed non-cholesterol diet without tu-chung, whereas fatty acid synthetase was not influenced (table 3). Tu-chung supplementation to the diet with cholesterol had no effect on ACC, FAS and 3-hydroxy-3-methylglutaryl-CoA reductase activities. Dietary cholesterol did not significantly increase ACC activity but it decreased FAS ($p<0.001$) and 3-hydroxy-3-methylglutaryl-CoA reductase activities ($p<0.001$). There was interaction between tu-chung leaf meal and cholesterol on ACC activity.

Hepatic triglyceride increased when tu-chung was added to the diet without cholesterol ($p<0.01$) but it decreased when tu-chung was added to the diet with cholesterol ($p<0.01$); phospholipids were not influenced (table 4). Dietary cholesterol significantly increased free cholesterol ($p<0.001$) and decreased triglyceride ($p<0.05$) in the liver with no effect on phospholipids. In comparison with a high-cholesterol diet without tu-chung, less plasma phospholipids ($p<0.05$), triglyceride ($p<0.01$), free cholesterol ($p<0.05$) and cholesterol ester ($p<0.05$) were found when tu-chung was added to diet with cholesterol (table 5). Tu-chung

Table 2. Effect of tu-chung supplementation to the diet with or without cholesterol on body weight, feed intake, feed conversion ratio and liver weight of chickens

Variable	Cholesterol (-)[1]		Cholesterol (+)		Analysis of variance		
	Tu-chung (-)[2]	Tu-chung (+)	Tu-chung (-)	Tu-chung (+)	Chol.	Tu-chung	Interaction
Initial BW (g)	879 ± 34[3]	880 ± 40	879 ± 35	879 ± 35	NS	NS	NS
Final BW (g)	1,280 ± 48	1,257 ± 61	1,258 ± 54	1,233 ± 39	NS	NS	NS
BWG (g)	401 ± 44	377 ± 35	380 ± 36	355 ± 31	NS	NS	NS
Feed intake (g)	1,552 ± 30	1,591 ± 35	1,546 ± 25	1,536 ± 32	NS	NS	NS
FCR	3.87 ± 0.8	4.22 ± 0.9	4.07 ± 1.1	4.33 ± 0.8	NS	NS	NS
Liver weight (% BW)	1.89 ± 0.06[4]	1.96 ± 0.10	2.39 ± 0.13	2.47 ± 0.06	p<0.001	NS	NS

[1] Diet was unsupplemented (-) or supplemented (+) with cholesterol.
[2] Diet was unsupplemented (-) or supplemented (+) with tu-chung.
[3] Mean ± SD for 10 chickens.
[4] Mean ± SD for 8 chickens.

Table 3. Effect of tu-chung supplementation to the diet with or without cholesterol on lipogenic and cholesterogenic enzyme activity in chickens

Variable	Cholesterol (-)[1]		Cholesterol (+)		Analysis of variance		
	Tu-chung (-)[2]	Tu-chung (+)	Tu-chung (-)	Tu-chung (+)	Chol.	Tu-chung	Interaction
			nmol/min/ mg protein				
Acetyl-CoA carboxylase	1.68 ± 0.203	2.40 ± 0.28**[4]	1.96 ± 0.22	1.90 ± 0.17	NS	p<0.001	p<0.001
Fatty acid synthetase	10.75 ± 1.31	10.75 ± 1.31	8.64 ± 1.21	7.98 ± 0.77	p<0.001	NS	NS
			pmol/min/ mg protein				
3-hydroxy-3-metyl-glutaryl-CoA reductase	86.35 ± 16.90	64.35 ± 12.47**	22.24 ± 3.23	19.75 ± 6.16	p<0.001	p<0.05	NS

[1] Diet was unsupplemented (-) or supplemented (+) with cholesterol.
[2] Diet was unsupplemented (-) or supplemented (+) with tu-chung.
[3] Mean ± SD for 8 chickens.
[4] Significantly different from the nil-cholesterol diet without tu-chung; ** p<0.01.

supplementation to the diet without cholesterol decreased plasma free cholesterol (p<0.05) with no effect on other lipid fractions. Dietary cholesterol significantly increased cholesterol ester, free cholesterol, triglyceride, and phosdpholipids in plasma (p<0.001).

DISCUSSION

The tendency of poor feed conversion ratio and lower body weight gain in chickens fed tu-chung indicated that tu-chung supplementation had no advantageous effect on growth characteristics. A high tannins content in tu-chung leaf meal might partly cause the tendency of poor feed conversion ratio and body weight gain. Poor feed conversion ratios and body weight gains attributable to tannins have been reported in a number studies (Armstrong et al., 1973; Douglas et al., 1990; Elkin et al., 1995). Muramatsu et al. (1993) found that in laying hens at a late production stage, tu-chung supplementation had no effect on feed intake and feed conversion ratio. It seems that growth phase could influence the response of chickens to tu-chung supplementation.

It is well documented that dietary cholesterol has a negative feedback on cholesterol synthesis in the liver (Dietschy and Wilson, 1976a, b) as well as hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity (Stage et al., 1981; Youn et al., 1993). The present results confirmed the above observation. Therefore, an increase in hepatic free cholesterol and plasma free cholesterol and cholesterol ester would be a result of cholesterol derived from diet. The present results show that 1% cholesterol feeding did not stimulate hepatic

Table 4. Effect of tu-chung supplementation to the diet with or without cholesterol on contents of lipid fractions in the liver of chickens

Variable	Cholesterol (-)[1]		Cholesterol (+)		*Analysis of variance*		
	Tu-chung (-)[2]	Tu-chung (+)	Tu-chung (-)	Tu-chung (+)	Chol.	Tu-chung	Interaction
	mg/g liver						
Free cholesterol	3.48±0.163	3.49±0.29	11.45±2.33	10.66±1.46	p<0.001	NS	NS
Triglyceride	3.23±1.10	6.06±2.38**[4]	5.45±2.68	2.01±0.34**	p<0.05	NS	P<0.001
Phospholipids	46.61±2.59	45.24±4.07	45.54±4.45	48.01±2.20	NS	NS	NS

[1] Diet was unsupplemented (-) or supplemented (+) with cholesterol.
[2] Diet was unsupplemented (-) or supplemented (+) with tu-chung.
[3] Mean±SD for 8 chickens.
[4] Significantly different from diet without tu-chung in the same cholesterol diet; * p<0.05, ** p<0.01.

Table 5. Effect of tu-chung supplementation to the diet with or without cholesterol on concentration of lipid fractions in the plasma of chickens

Variable	Cholesterol (-)[1]		Cholesterol (+)		Analysis of variance		
	Tu-chung (-)[2]	Tu-chung (+)	Tu-chung (-)	Tu-chung (+)	Chol.	Tu-chung	Interaction
	mg/100 ml						
Cholesterol ester	127.73± 9.05[3]	117.92±11.58	961.12±97.53	845.87±12.88*	p<0.001	p<0.05	NS
Free cholesterol	34.67± 1.72	31.77± 2.80*	197.31± 8.68	171.4 ±24.69*	p<0.001	p<0.001	p<0.05
Triglyceride	102.45±16.87	108.45±27.57	196.25±39.59	141.41±19.11**	p<0.001	p<0.05	p<0.001
Phospholipids	336.44±34.18	301.46±36.82	483.71±20.83	444.35±28.92*	p<0.001	p<0.001	NS

[1] Diet was unsupplemented (-) or supplemented (+) with cholesterol.
[2] Diet was unsupplemented (-) or supplemented (+) with tu-chung.
[3] Mean±SD for 10 chickens.
[4] Significantly different from diet without tu-chung in the same cholesterol diet; * p<0.05, ** p<0.01.

fatty acid synthesis as indicated by no change in hepatic ACC activity. This observation did not agree with the observation of Fungwe et al. (1994) who found that dietary cholesterol stimulated fatty acid synthesis in rats. No change in hepatic fatty acid synthesis would result in no change in hepatic triglyceride. The current study, however, shows that dietary cholesterol resulted in lower hepatic triglyceride. Fungwe et al. (1994) found that there was evidence that livers from cholesterol-fed rats secrete triglyceride more rapidly. This evidence might partly explain lower hepatic triglyceride. Higher triglyceride secretion into the bloodstream might partly explain higher plasma cholesterol in the current study. No increased incorporation of newly synthesized fatty acid into hepatic phospholipids in liver from cholesterol-fed rats (Fungwe et al., 1994) might explain no change in liver phospholipids in the present study. However, it remains unknown why cholesterol feeding increased plasma phospholipid in the present study.

A decrease in plasma free cholesterol by tu-chung supplementation in chickens fed the diet without cholesterol may result from a decrease in hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity, a rate-limiting enzyme in cholesterol synthesis. It is unknown why tu-chung supplementation in chickens fed the diet without cholesterol increased hepatic ACC and triglyceride with no increase in plasma triglyceride. It is possible that this diet resulted in higher triglyceride clearance. It is interesting to note that there was interaction of tu-chung and cholesterol feeding on ACC activity, hepatic and plasma triglyceride. Tu-chung leaf meal increased ACC activity, hepatic and plasma triglyceride when it was added to the diet without cholesterol, but it reduced ACC activity, hepatic and plasma triglyceride when it was added to the diet with cholesterol. Thus, tu-chung leaf meal has hypotriglyceride properties if added to a diet with cholesterol, but it has hypertriglyceride properties if added to a diet without cholesterol.

A slight decrease in 3-hydroxy-3-methylglutaryl-CoA reductase activity caused by tu-chung supplementation in the liver of chickens fed the diet with cholesterol might not fully explain a decrease in plasma free cholesterol. It is of interest to note that a decrease in liver and plasma triglyceride with this diet was not accompanied by lower hepatic lipogenic enzyme activities, leading to assumption of higher

triglyceride clearance.

The present study disagrees with the observation of Muramatsu et al. (1993), who found no change in plasma cholesterol of laying hens, but agrees with the observation of Ohashi et al. (1991) who found that in rats, serum cholesterol was lowered by supplementing a high-cholesterol diet with tu-chung leaf meal. Therefore, it is likely that growth phase will influence the response of chickens to tu-chung leaf meal.

Which component in tu-chung leaf meal affects hepatic lipogenic and cholesterogenic enzyme activities as well as plasma lipid fraction concentration is still unknown. As shown in table 1, tu-chung leaf meal is rich in dietary fiber (pectin) and tannin. Fisher et al. (1966) reported that pectin reduced serum cholesterol and retarded atherosclerosis. Akiba and Muramatsu (1978, 1980) showed that dietary fibers reduced liver lipid deposition and plasma lipid content in growing chickens. In addition, pectin and other dietary fibers are known to reduce apparent fat digestibility (Deuchi et al., 1994). Tannin is also known to reduce serum cholesterol concentration (Muramatsu et al., 1986) and serum triglyceride VLDL and cholesterol, and increase HDL-cholesterol (Tebib et al., 1994). Therefore, it is assumed that tannin and pectin (crude fiber) in tu-chung leaf meal might contribute to lowering plasma cholesterol and triglyceride in chickens. It is concluded that, tu-chung leaf meal might have active compounds that could reduce plasma triglyceride and cholesterol accretion stimulated by cholesterol supplementation. In addition, tu-chung leaf meal supplementation to the diet without cholesterol increased triglyceride in the liver, therefore causing higher hepatic fat accumulation.

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