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The Effects of White Kwao Krua (*Pueraria mirifica*) on Serum Lipid Profile, Egg–Yolk Cholesterol Levels, and Egg Production in Laying Hens

Prapassara Kanpai[1], Vorasak Patchimasiri[1], Soontaranee Tongyai[1], Arunee Engkagul[2] and Chanin Tirawattanawanich[1]

ABSTRACT

Lipid metabolisms in layers are mainly controlled by estrogen. Therefore, administration of White Kwao Krua (*Pueraria mirifica*, PM) containing potent phytoestrogen may influence serum lipid components and egg yolk cholesterol. A randomized complete block designed experiment was conducted using a total of 80, 18-week-old laying hens. The hens were equally divided into 4 groups receiving 4 different treatment diets containing PM at 0, 100, 500 and 1,000 ppm. Daily egg production was recorded. Serum samples were collected at week 0, 2, 4, 6, 8 and 17 of the treatment for determination of lipid components and estradiol. Egg samples were collected at week 4 and 8, yolk cholesterol was extracted and measured. No significant differences in egg production pattern were found. At week 4 of the treatment, the control group showed significantly higher serum triglyceride and total cholesterol levels compared to other treatment groups ($P<0.05$), and a lower level of serum high density lipoprotein (HDL) compared to the group fed 1,000 ppm PM fortified diets ($P<0.05$). Serum estrogen levels were found significantly lower in groups fed PM fortified diets at week 8 ($P<0.05$) compared to the control. No significant differences in egg yolk cholesterol levels were detected among the treatment groups.

Key words: *Pueraria mirifica*, serum lipid profile, laying hens

INTRODUCTION

Control of dietary cholesterol level is one important means to reduce risk of atherosclerosis, a significant etiological factor of Coronary Heart Disease (CHD) causing death and disability (Ariyo and Villablanca, 2002). Risk factors of disease include age (Witteman *et al*., 1989), family history (Ordovas *et al*., 2000), types of food and eating habits (Schaefer, 2002). Egg yolk is always of major concern due to its high cholesterol content (Feeley *et al*., 1972). Although research showed that egg consumption has only little effect on blood cholesterol (Page *et al*., 1991), people who are at risk should take precaution. Availability of low-cholesterol eggs will provide a source of high quality protein at low cost and healthy for most human (Richards, 1997).

It has been found that estrogen can reduce blood cholesterol (Mendelsohn and Karas, 1999), which may affect the cholesterol supply for yolk synthesis. PM containing potent phytoestrogen is therefore studied for the effect on serum lipid components, egg production, and egg yolk cholesterol in laying hens.

1 Department of Physiology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
2 Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

MATERIALS AND METHODS

Animals and treatments: Eighty, 18-week-old Newscommatt laying hens were randomly divided into 4 groups receiving 4 different treatment diets for 22 weeks. The treatment diets included corn-soybean basal diet containing PM at 0 (control), 100, 500 and 1,000 ppm. Daily egg production was recorded.

Samples collection: Serum samples were collected at week 0, 2, 4, 6, 8 and 17 of the treatments and stored at -20°C for later examination of lipid components and estradiol. Egg samples were collected at week 4 and 8 for the examination of the whole egg and yolk weights and for yolk cholesterol analysis.

Analytical procedure: Serum estradiol concentrations were measured using microparticle enzyme immunoassay (JDH Borneo Limited, Thailand). Serum lipid profiles were quantified by enzymatic assay using commercially available kits (Human, Germany) and analysed spectrophotometrically at the wavelength of 500 nm. Egg yolk cholesterol was determined using the method modified from Shen *et al*. (1982). Briefly, total lipid in 1 g of egg yolk was extracted by methylene chloride-methanol (2:1, v/v). The lipid extract was then saponified with methanolic sodium hydroxide, and finally, free cholesterol was extracted using petroleum ether. The cholesterol extract was analysed spectrophotometrically at the wavelength of 500 nm. Results were reported as means±SEM.

Statistical analysis: Differences among means were analysed by one-way ANOVA. Duncan's new multiple range test was used to compare between control and treatments. Significant differences were justified at $P<0.05$.

RESULTS AND DISCUSSION

Table 1 shows significant increases of serum triglyceride levels in all treatment groups up to week 8 of treatments ($P<0.05$). No significant differences were found within each treatment when compared between week 8 and 17 except for the 1,000 ppm PM treatment in which an increase of triglyceride was still detected ($P<0.05$). Serum estradiol also increased significantly from the beginning to week 8 of treatments (Table 2), which corresponded to the increase of egg production (Figure 1). Peak production was found at week 8 of treatments. These results confirmed the positive relationships of serum estradiol, serum triglycerides and egg production. Increased estradiol in the blood circulation corresponds to

Table 1 Effects of PM supplementation on serum triglycerides.

Weeks of treatment	Serum triglycerides (mg/dl)			
	Treatment 1 [2/] (0 ppm PM)	Treatment 2 [2/] (100 ppm PM)	Treatment 3 [2/] (500 ppm PM)	Treatment 4 [2/] (1,000 ppm PM)
0 [1/]	314.90±229.89 [a,C]	240.49±143.12 [a,C]	320.76±273.37 [a,C]	321.85±267.23 [a,D]
2 [1/]	848.70±225.28 [a,B]	680.20±358.33 [a,B]	598.90±355.57 [a,C]	721.50±350.63 [a,C]
4 [1/]	1151.20±491.47 [a,A]	989.20±228.58 [b,B]	1119.70±431.40 [b,B]	1004.50±390.78 [b,C]
6 [1/]	1671.10±769.67 [a,A]	1378.30±517.19 [a,A]	1403.00±499.14 [a,AB]	1397.10±525.63 [a,B]
8 [1/]	1809.50±716.76 [a,A]	1684.10±625.89 [a,A]	1529.40±608.97 [a,A]	1537.10±479.21 [a,B]
17 [1/]	1805.60±647.44 [a,A]	1527.40±452.54 [a,A]	1512.80±340.17 [a,A]	1866.00±472.33 [a,A]

[1/] Means in the same row with the different small letter superscripts are significantly different ($P<0.05$).

[2/] Means in the same column with the different capital letter superscripts are significantly different ($P<0.05$).

good follicular development and consecutive ovulation and egg production. Estradiol also influences lipid metabolism. Mechanisms were found associated with the stimulation, at high dose, of hormone sensitive lipase and reduction of lipoprotein lipase synthesis resulting in lipolysis in human (Palin *et al*., 2003). The lypolytic effect was also reported in estrogenized chickens in which the endogenous hypertriglyceridemia was observed (Kudzma *et al*., 1975). However estrogen at low dose was found to promote lipogenesis by stimulating lipoprotein lipase synthesis. Other estrogen functions were also found associated with the reduction of liver synthesis and secretion of apoA-I (a major apolipoprotein component of HDL) in the chicken hepatoma cell line, LMH-2A (Hermann *et al*., 2003), the inhibition of apoA-I transcription in rats (Taylor *et al*., 2003), the increase of apoB-100 (Bujo *et al*., 1997), and the induction of apoB synthesis in the cockerel liver (Chan *et al*., 1976). This effected on the decrease of HDL and the increase of VLDL and yolk-

Table 2 Effects of PM supplementation on serum estradiol.

Weeks of treatment	Serum estradiol (pg/ml)			
	Treatment 1 2/ (0 ppm PM)	Treatment 2 2/ (100 ppm PM)	Treatment 3 2/ (500 ppm PM)	Treatment 4 2/ (1,000 ppm PM)
0 1/	142.10±62.62 a,B	149.08±54.32 a,B	155.08±60.79 a,C	132.10±67.20 a,B
2 1/	188.73±68.76 a,B	190.28±52.85 a,B	170.88±68.62 a,C	167.69±67.45 a,B
4 1/	324.88±73.57 a,A	286.25±57.11 a,A	284.34±31.57 a,AB	297.61±37.50 a,A
6 1/	324.96±38.67 a,A	312.57±45.09 a,A	319.75±51.63 a,AB	308.16±46.43 a,A
8 1/	327.88±37.20 a,A	311.44±55.96 ab,A	264.11±104.13 b,B	285.47±50.91 ab,A
17 1/	333.67±65.41 a,A	305.73±95.51 a,A	331.40±83.87 a,A	319.85±65.50 a,A

1/ Means in the same row with the different small letter superscripts are significantly different (P<0.05).
2/ Means in the same column with the different capital letter superscripts are significantly different (P<0.05).

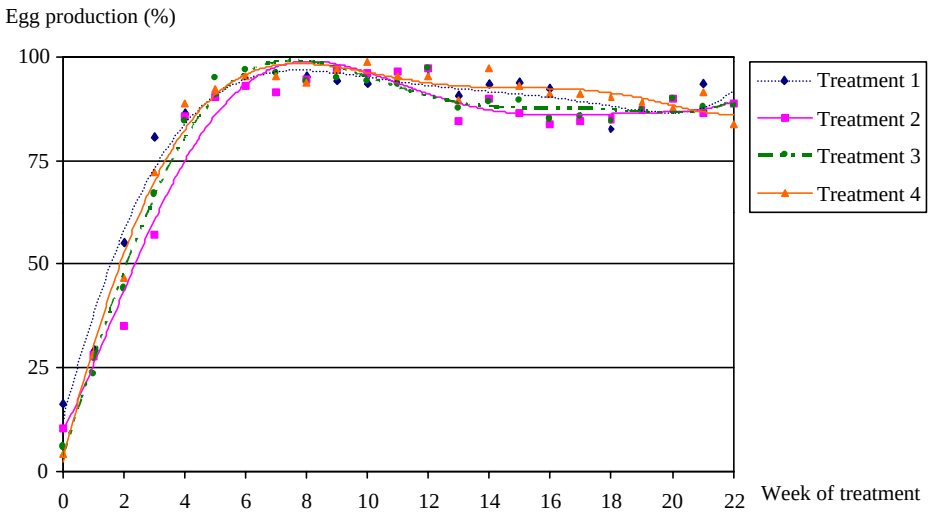

Figure 1 Effects of PM supplementation on egg production.

targeted VLDL (VLDLy). VLDLy is more compact than VLDL. Its transportation across oocyte membrane was found to be by means of receptor-mediated endocytosis (Walzem *et al*., 1999).

When compared among treatment groups, the control showed the higher level of serum triglyceride and cholesterol at week 4 of treatments ($P<0.05$) (Table 3). Although the differences in other weeks were not statistically significant, means of serum triglyceride and cholesterol levels tended to be higher in the control group as well. Results also showed that all groups fed PM fortified diets seem to have higher serum HDL levels, but only the group fed 1,000 ppm PM fortified diets at week 4 have significantly higher serum HDL levels when compared to the control ($P<0.05$) (Table 4). Similar effects on serum lipid components have been reported in menopausal women (Wangen *et al*., 2001) and ovariectomized cynomolgus monkeys after being treated with isoflavone, a phytoestrogen (Greaves *et al*., 2000). Such effect of PM in mice was also reported in which reduced serum triglyceride, cholesterol, and low density lipoprotein (LDL), and elevated HDL was stated (Chivaput *et al*., 2000). Egg yolk cholesterol levels, however, were not different in all treatment groups

Table 3 Effects of PM supplementation on serum cholesterol.

Weeks of treatment	Serum cholesterol (mg/dl)			
	Treatment 1 2/ (0 ppm PM)	Treatment 2 2/ (100 ppm PM)	Treatment 3 2/ (500 ppm PM)	Treatment 4 2/ (1,000 ppm PM)
0 1/	127.79±27.98 a,A	118.74±11.62 a,B	128.69±29.11 a,A	102.62±53.02 a,B
2 1/	124.13±20.98 a,A	122.39±22.16 a,B	129.77±44.80 a,A	122.40±32.07 a,AB
4 1/	149.14±43.15 a,A	115.15±27.98 b, B	117.24±21.41 ab,A	123.06±25.02 ab,AB
6 1/	146.28±56.29 a,A	129.51±28.73 a,AB	129.50±25.64 a,A	133.92±34.32 a,A
8 1/	149.71±38.81 a,A	147.52±37.39 a,A	137.89±29.16 a,A	135.73±27.10 a,A
17 1/	150.31±44.74 a,A	136.77±20.63 a,AB	132.02±20.51 a,A	150.47±25.03 a,A

1/ Means in the same row with the different small letter superscripts are significantly different ($P<0.05$).

2/ Means in the same column with the different capital letter superscripts are significantly different ($P<0.05$).

Table 4 Effects of PM supplementation on serum HDL.

Weeks of treatment	Serum HDL (mg/dl)			
	Treatment 1 2/ (0 ppm PM)	Treatment 2 2/ (100 ppm PM)	Treatment 3 2/ (500 ppm PM)	Treatment 4 2/ (1,000 ppm PM)
0 1/	41.80±19.27 a,A	44.79±14.73 a,A	43.89±22.57 a,A	50.07±64.12 a,A
2 1/	14.67±9.35 a,B	18.80±10.09 a,B	24.64±18.61 a,B	21.29±13.45 a,B
4 1/	7.73±3.86 b,BC	10.00±3.47 ab,C	9.58±3.12 ab,C	11.54±4.65 a,B
6 1/	5.93±2.40 a,C	4.94±3.07 a,C	5.19±2.79 a,C	6.24±2.69 a,B
8 1/	4.96±2.33 a,C	6.43±2.19 a,C	5.66±2.18 a,C	6.86±3.10 a,B
17 1/	5.72±2.94 a,C	6.66±3.59 a,C	5.99±2.78 a,C	5.41±2.69 a,B

1/ Means in the same row with the different small letter superscripts are significantly different ($P<0.05$).

2/ Means in the same column with the different capital letter superscripts are significantly different ($P<0.05$).

Table 5 Effects of PM supplementation on egg yolk cholesterol.

Weeks of treatment	Egg yolk cholesterol (mg/g yolk)			
	Treatment 1 2/ (0 ppm PM)	Treatment 2 2/ (100 ppm PM)	Treatment 3 2/ (500 ppm PM)	Treatment 4 2/ (1,000 ppm PM)
4 1/	12.64±1.54 a,A	12.74±1.97 a,A	14.21±3.37 a,A	13.43±1.95 a,A
8 1/	11.76±1.17 a,A	11.29±1.10 a,A	11.31±0.77 a,B	11.31±1.58 a,B

1/ Means in the same row with the different small letter superscripts are significantly different (P<0.05).

2/ Means in the same column with the different capital letter superscripts are significantly different (P<0.05).

(Table 5). This suggested that other factors may play a role in the deposition of cholesterol and other lipid components in eggs (Walzem *et al*., 1999).

The effects of PM on the alteration of serum lipid components in layers found in this experiment might be partly explained by the function through negative feedback control of endogenous estradiol. PM has been found to suppress follicle stimulating hormone (FSH) secretion in cynomolgus monkeys (Malaivijitnond *et al*., 2002). This negatively affects on follicle maturation resulted in decreasing of estrogen synthesis and secretion. Reduced circulating estrogen reported herein may minimize the effect on the above mentioned lipid metabolism thereby reducing serum triglyceride while increasing HDL. Besides the action through estrogen negative feedback control, PM may increase HDL levels by directly stimulating liver synthesis of apoA-I and apoA-II, which was reported as a function of isoflavone (Clarkson and Anthony, 1998). Soy phytoestrogens including genistein and daidzein, have been found to increase plasma levels of HDL and apoA-I in the human hepatoma cell line, Hep G2 (Lamon-Fava, 2000). These findings suggested an atherosclerotic risk reducing role in human.

In conclusion, this experiment has demonstrated some significant effects of PM on serum lipid components in layers. This could lead to the application of PM to manipulate lipid metabolism to either improve energy utilization efficiency, productions or solve metabolic associated problems in laying hens. Detailed mechanisms are however remained to be scrutinized.

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