# Effects of Feeding Egg Yolk Prepared from Quails Fed Winged Bean Oil on Plasma and Liver Cholesterol and Fatty Acid Composition of Rats

**R. Mutia and S. Uchida***
Faculty of Agriculture, Okayama University, Tsushima naka 1-1-1, Okayama 700, Japan

**ABSTRACT** : The purpose of this experiment was to study the effects of feeding egg yolk prepared from quail fed winged bean oil on plasma and liver cholesterol and fatty acids composition in rats. Eggs were collected from laying quails that had been fed diets containing 5% of either animal tallow, soybean oil, corn oil or winged bean oil. Yolk powders (referred to as winged bean yolk, soybean yolk, etc.) were prepared and fed at the 15% level to weanling female Wistar rats for 28 days. Rats fed winged bean yolk had significantly lower body weight gain than did rats fed soybean yolk or animal tallow yolk. There are no significant effects on plasma total cholesterol, liver cholesterol and triglyceride concentration due to feeding yolk powder containing different oil. However, LDL-cholesterol increased and HDL-cholesterol decreased in rats fed winged bean yolk. Liver lipid of rats fed with winged bean yolk had a higher linoleic but a lower linolenic content than did those fed animal tallow yolk. However, rats fed with winged bean yolk had a lower linoleic content than did rats fed soybean yolk or corn yolk. In conclusion, winged bean yolk seemed to have had an adverse effect on rats. *(Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 2 : 192-196)*

**Key Words** : Winged Bean Oil, HDL-Cholesterol, LDL-Cholesterol, Liver Fatty Acids

## INTRODUCTION

The winged bean (*Psophocarpus tetragonolobus*) is considered to be a promising source of protein and oil in the humid tropics where it is native, grows well, and tolerates a wide range of altitude (NAS, 1981). Winged bean seeds have high levels of protein and oil ranging from 27.8~47.2% and 15.2~27.8%, respectively (Khor et al., 1982; Okezie and Martin, 1980). The fatty acid composition of winged bean is similar to that of peanut oil. Total unsaturated fatty acids account for 65% of the total fatty acids (Khor et al., 1982). Winged bean oil not only contains acceptable amounts of unsaturated fatty acid, especially linoleic acid, but in contrast to soybean oil, the content of linolenic acid is quite low, giving winged bean oil the advantage of greater stability. Winged bean oil also contains a high measure of tocopherol. Some varieties are reported to have levels higher than those of soybean or corn oil (Cerney et al., 1971; de Lumen and Fiad, 1982). Winged bean oil contains behenic acid, as does peanut oil, but although this may reduce its digestibility, it seem unlikely to have other ill effects (Cerney et al., 1971; Khor et al., 1982; NAS, 1981).

In our previous experiment, feeding a diet containing 5% winged bean oil did not impair laying performance of Japanese quails. However, the cholesterol content of eggs produced with the winged bean oil diet was higher than the cholesterol content of eggs produced with soybean oil or tallow diet. Egg oleic content was higher and egg linoleic content was lower with winged bean oil diet than with the soybean or corn oil diets. To better understand the effects of winged bean oil in the egg, we investigated the effect of feeding a diet containing these eggs on plasma and tissue cholesterol and fatty acid composition of rats.

## MATERIALS AND METHODS

### Laying quails diet and yolk powder preparation

Winged bean seeds were obtained from cultivars that are locally grown in Indonesia. Seeds were milled, and then the oils were extracted with ether in a soxhlet apparatus for 6 h. Four diets were prepared by mixing 5% of either tallow, soybean oil, corn oil (purchased from commercial sources) or winged bean oil with a formulated basal diet that meets NRC (1994) requirements. The compositions of the experimental diets are presented in table 1.

**Table 1.** Composition of Japanese quails diets

| Ingredients | Amounts (%) | | |
|---|---|---|---|
| Corn | 38.8 | *Calculated analyses :* | |
| Soybean meal | 29.0 | Crude Protein (%) | 20.3 |
| Wheat | 20.0 | ME (kcal/kg) | 2,890 |
| Oil[1] | 5.0 | Ca (%) | 2.6 |
| $CaCO_3$ | 2.0 | Total P (%) | 0.4 |
| DL-Methionine | 0.5 | Fat (%) | 2.2 |
| Choline Chloride | 0.2 | Lysine (%) | 1.0 |
| Mineral mix[2] | 3.5 | | |
| Vitamin mix[3] | 1.0 | | |

[1] Each treatment contained 5% of either animal tallow, soybean oil, corn oil or winged bean oil.
[2] Mineral mix contained per kg diet: $CaHPO_4$, 1.75%; NaCl, 0.252%; $K_3C_6H_5O_7.H_2O$, 0.77%; $K_2SO_4$, 0.182(%); MgO, 840 mg; $MnCO_3$ (43-48% Mn), 122.5 mg; Fe-Citrate (16-17% Fe), 210 mg; $ZnCO_3$ (70% Zn), 56 mg; $CuCO_3$ (53-55% Cu), 10.5 mg; $KIO_3$, 0.7 mg; $Na_2SeO_3$, 0.25 mg; $CrK(SO_4)_2 \cdot 12H_2O$, 19.25 mg.
[3] Vitamine mix contained per kg diet: Thiamine, 6 mg; Riboflavin, 6 mg; Piridoxin, 7 mg; Nicotinic acid, 30 mg; D-Pantotenic acid calcium, 16 mg; D-Biotine, 0.2 mg; Cyanocobalamine, 0.01 mg; Vit. A (Retinol), 4000 IU; DL-Tocopherol, 50 IU; Vit. $D_3$, 1000 IU; Vit. $K_3$, 0.05 mg.

* Address reprint request to S. Uchida
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Ten 10-week-old Japanese quail were used for each treatment. The birds were placed in individual cages in a constant temperature room (25℃) and kept on a 14L:10D h of light-dark cycle. Birds were fed fresh daily. Feed and water were made available *ad libitum*. From each treatment, eggs laid during 8 weeks feeding trial were collected. The eggs were hard-boiled, and the yolks were separated, pulverized and dried at 50℃ for 3 h. The fatty acid compositions of the yolk powder preparations were determined by gas chromatography (table 2).

**Table 2.** Major fatty acids of yolk powder

| Fatty acids (%) | Treatments[1] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Myristic | 0.37 | 0.17 | 0.19 | 0.24 |
| Palmitic | 31.67 | 31.49 | 31.35 | 31.11 |
| Palmitoleic | 3.37 | 1.96 | 1.89 | 2.79 |
| Stearic | 14.29 | 18.47 | 17.69 | 16.32 |
| Oleic | 38.48 | 25.27 | 27.62 | 32.80 |
| Linoleic | 10.84 | 21.10 | 19.52 | 14.55 |
| Linolenic | 0.23 | 0.91 | 0.48 | 0.58 |
| Arachidonic | 0.75 | 0.62 | 1.27 | 1.61 |
| SFA | 46.33 | 50.13 | 49.23 | 47.67 |
| MUFA | 41.85 | 27.23 | 29.51 | 35.59 |
| PUFA | 11.82 | 22.63 | 21.27 | 16.74 |

The values presented are means of duplicate determinations.
[1] A=basal diet+5% animal tallow, B=basal diet+5% soybean oil, C=basal diet+5% corn oil, D=basal diet+5% winged bean oil.
SFA=saturated fatty acids, MUFA=mono-unsaturated fatty acids, PUFA=poly-unsaturated fatty acids.

### Animals and diets

Twenty eight female Wistar rats, 3 wk of age, were housed individually in wire cages in a constant temperature room (25℃) and received 14L:10D h of light-dark cycle. After feeding a commercial diet (CLEA Japan Inc.) for 5 days, they were divided into four groups with seven rats each, in such a manner that the mean body weight of each group was similar.

Four diets were prepared by mixing 85% basal diet mix with 15% egg yolk powders (table 3). To prevent lipid peroxidation, the diets were mixed every week and stored in sealed containers at 4℃. Rats were fed fresh daily. Feed and water were made available *ad libitum* .

### Sample collection and analyses

At the end of the 28-day feeding trial, rats were fasted overnight and sacrificed by decapitation the following morning. Blood samples was collected into heparinized tubes and centrifuged at 4℃ at 3000 rpm for 15 min to separate the plasma. Rat liver tissue was quickly excised, blotted dry, weighed, and stored at -20℃ for lipid analyses. Plasma total cholesterol was analyzed by an enzymatic method using a commercial kit (Sterozyme-545). Plasma triglyceride and HDL-cholesterol were analyzed by enzymatic method using a commercial kit (Wako Pure Chemical Ind. Co., Osaka). LDL-cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol.

**Table 3.** Composition of rat diets

| | Treatments[1] | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Ingredients (%) | | | | |
| Casein | 19.0 | 19.0 | 19.0 | 19.0 |
| Yolk powder | 15.0 | 15.0 | 15.0 | 15.0 |
| Corn starch | 36.0 | 36.0 | 36.0 | 36.0 |
| Glucose | 20.0 | 20.0 | 20.0 | 20.0 |
| DL-Methionine | 0.3 | 0.3 | 0.3 | 0.3 |
| Choline chloride | 0.2 | 0.2 | 0.2 | 0.2 |
| Cellulose | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamin mix[2] | 1.0 | 1.0 | 1.0 | 1.0 |
| Mineral mix[3] | 3.5 | 3.5 | 3.5 | 3.5 |
| Proximate analysis: | | | | |
| Crude Protein (%) | 21.2 | 21.5 | 21.2 | 20.7 |
| Crude Fat (%) | 10.4 | 10.3 | 10.8 | 9.2 |

[1] A=basal diet+15% tallow yolk; B=basal diet+15% soybean yolk; C=basal diet+15% corn yolk; D=basal diet+15% winged bean yolk.
[2] Vitamine mix contained per kg diet: Thiamine, 6 mg; Riboflavin, 6 mg; Piridoxin, 7 mg; Nicotinic acid, 30 mg; D-Pantotenic acid calcium, 16 mg; D-Biotine, 0.2 mg; Cyanocobalamine, 0.01 mg; Vit. A (Retinol), 4000 IU; DL-Tocopherol, 50 IU; Vit. $D_3$, 1000 IU; Vit. $K_3$, 0.05 mg.
[3] Mineral mix contained per kg diet: $CaHPO_4$, 1.75%; NaCl, 0.252%; $K_3C_6H_5O_7.H_2O$, 0.77%; $K_2SO_4$, 0.182(%); MgO, 840 mg; $MnCO_3$ (43-48% Mn), 122.5 mg; Fe-Citrate (16-17% Fe), 210 mg; $ZnCO_3$ (70% Zn), 56 mg; $CuCO_3$ (53-55% Cu), 10.5 mg; $KIO_3$, 0.7 mg; $Na_2SeO_3$, 0.25 mg; $CrK(SO_4)_2 \cdot 12H_2O$, 19.25 mg.

Total lipid from liver was extracted using chloroform:methanol (2:1, vol/vol) according to the method of Folch et al. (1957). Liver lipid was determined gravimetrically. Methyl esters of the lipid extract were prepared according to Morrison and Smith (1974). Fatty acid composition of liver lipid was determined using a gas chromatograph (Shimadzu GC-14A) equipped with a capillary column (50 m by 0.25 mm inside diameter) and an integrator (Shimadzu C-R6A Chromatopac). The samples were chromatographed at 150 to 220℃ with a 3℃/min programmed temperature gradient. Injector and detector temperatures were set at 250℃, and helium was used as a carrier gas. Fatty acid methyl esters were identified by comparison of retention times with known standards and expressed as percentages of methyl esters. For cholesterol determination, lipid from liver samples was extracted by the method of Folch (1957). The samples were saponified by the method of Abell (1952) as modified by Beyer (1989). The concentration of cholesterol was determined enzymatically using a commercial kit (Sterozyme-545).

### Statistical analysis

Analysis of variance was carried out on data using the General Linear Models (GLM) procedure. Significant differences of means were tested using the LSD (Least Significant Difference) method and significance was declared at $p<0.05$.

## RESULTS AND DISCUSSION

### Fatty acid profile of yolk lipid

In the following, yolk powder from quails fed the different diets are referred to as winged bean yolk, soybean yolk, corn yolk, and tallow yolk. The fatty acid compo- sition of yolk powder was influenced by dietary fat source (table 2). Winged bean yolk contained higher myristic, palmitoleic, oleic and arachidonic than did soybean or corn yolk. However, stearic and linoleic content were lower in winged bean yolk than with soybean or corn yolk. On the other hand, winged bean yolk had lower contents of myristic, palmitoleic and oleic acids, but higher content of stearic, linoleic, linolenic and arachidonic acids than did animal tallow yolk. These results are consistent with the results of other reports that yolk fatty acid composition was easily altered by diet manipulation (Hargis et al., 1991; Cherian and Sim, 1991; Jiang et al., 1991).

### Rat performance and organ weight

Feeding winged bean yolk to rats did not affect their daily feed consumption. However, body weight gain of rats fed winged bean yolk lower ($p<0.05$) than those rats fed soybean or animal tallow yolk (table 4).

**Table 4.** Effects of dietary treatments on initial body weight, body weight gain, feed intake and liver weight of rats

| Response variable | Treatments[1] | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Initial body weight (g) | 58.21 | 58.49 | 58.77 | 58.43 | 1.37 |
| Body weight gain (g/day) | 5.03[a] | 5.02[a] | 4.70[b] | 4.76[b] | 0.11 |
| Feed intake (g/day) | 11.81 | 12.09 | 11.53 | 11.90 | 0.36 |
| Liver weight (g/100g BW) | 3.72 | 3.83 | 3.74 | 3.84 | 0.09 |

a,b Means (n=7) in same row with different superscript differ significantly ($p<0.05$).

1 A=basal diet+15% tallow yolk, B=basal diet+15% soybean yolk, C=basal diet+15% corn yolk, D=basal diet+15% winged bean yolk.

Since each of the diets had the same nutrient except for yolk powder, the possible explanation for these results is that the soybean yolk or animal tallow yolk are more digestible than winged bean and corn yolk. The differences in digestibility might be due to the different fatty acid compositions of the egg yolks. Fukushima et al. (1996) reported that rats fed mixture oil (palm oil, high-oleic safflower oil, and perilla oil) had a greater body weight gain than did rats fed other vegetable oil treatments. Another experiment with Japanese quails showed that quails fed a safflower oil diet had a heavier body weight than those quails fed on either linseed oil or tuna oil (Hood, 1991). Their reports showed that different fatty acid composition in the lipid used, would cause different response in body weight of experimental animals.

There are no significant differences in liver weight among the treatments.

### Plasma and tissue lipid

Plasma total cholesterol, liver cholesterol and plasma triglyceride levels were not significantly different among dietary treatments (table 5). However, plasma HDL-cholesterol concentration in rats fed winged bean yolk was lower ($p<0.05$) than it was in rats fed the soybean or animal tallow yolk, but was not significantly differ with corn yolk. On the other hand, plasma LDL-cholesterol concentration was higher ($p<0.05$) in rats fed the winged bean yolk than it was in rats fed the soybean or animal tallow yolk.

**Table 5.** Effects of dietary treatments on plasma cholesterol, liver cholesterol, HDL, LDL and triglyceride

| Response variable | Treatments[1] | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Plasma cholesterol (mg/dl) | 139.26 | 148.55 | 164.23 | 176.83 | 13.60 |
| HDL cholesterol (mg/dl) | 63.07[a] | 57.60[a] | 45.84[ab] | 32.70[b] | 6.48 |
| LDL cholesterol (mg/dl) | 76.19[b] | 90.95[b] | 118.39[ab] | 144.13[a] | 13.53 |
| Triglyceride (mg/dl) | 220.62 | 217.93 | 232.13 | 194.32 | 33.55 |
| Liver cholesterol (mg/g) | 34.01 | 33.66 | 36.45 | 34.43 | 3.86 |

a,b Means (n=7) in same row with different superscript differ significantly ($p<0.05$).

1 A=basal diet+15% tallow yolk, B=basal diet+15% soybean yolk, C=basal diet+15% corn yolk, D=basal diet+15% winged bean yolk.

Plasma LDL-cholesterol is mostly removed by receptor-dependent transport processes. Saturated fatty acids routinely decrease receptor activity whereas unsaturated fatty acids increase receptor-dependent LDL transport. The effect of saturated fatty acids on plasma LDL-cholesterol concentration, however, is always much greater than changes associated with manipulation of the unsaturated fatty acid content of the diet (Paik and Blair, 1996). In our experiment, winged bean yolk had higher poly unsaturated fatty acids (PUFA) but lower mono unsaturated fatty acids (MUFA) than animal tallow yolk. On the other hand, winged bean yolk had lower PUFA but higher MUFA than soybean yolk or corn yolk. Our results showed that, although winged bean yolk had higher PUFA than animal tallow yolk, rats fed the winged bean yolk had a higher LDL-cholesterol concentration and a lower HDL-cholesterol. Fukushima et al. (1996) reported that rats fed *Oenothera biennis* Linn oil, which contained 71% linoleic acid and 13.7% linolenic acid, had a lower LDL-cholesterol concentration than did rats fed other vegetable oils. Furthermore, they reported that rats fed palm oil, which contained 48.8% palmitic acid, 33.9% oleic acid, and 16.9% linoleic acid, had a higher HDL-cholesterol concentration than did rats fed other vegetable oils. However, Sugano et al. (1986) reported that olive oil containing less PUFA and more saturated fatty acid reduced the HDL-cholesterol concentration. Paik and Blair (1996) in a review reported that African monkeys given diets enriched in PUFA, either from safflower oil (n-6) or fish oil (n-3), had generally lower total plasma LDL and HDL-cholesterol concentration compared with monkeys given saturated fat diets. Both types of PUFA appeared to lower the concentration of the favorable cholesterol (HDL), while the effect to lower the unfavorable cholesterol (LDL) was also present. Furthermore, they reported that peanut oil was surprisingly atherogenic for rats, rabbits, and monkeys. This effect is not related to the level of long chain saturated fatty acids (arachidic, behenic, lignoceric) present in peanut oil, but rather to the oil's triglyceride structure. Randomization of peanut oil, which modifies its triglyceride structure, significantly reduces its atherogenicity. Winged bean oil, like penut oil, also has long chain saturated fatty acids. In our experiment, the increase in LDL-cholesterol in rats fed the winged bean yolk may be due to triglyceride structure of winged bean oil. However, further studies will be needed to determine whether winged bean oil is atherogenic for animals.

**Fatty acid of liver lipid**

Fatty acid composition of rat liver lipid is summarized in table 6. The contents of myristic, palmitic, palmitoleic, stearic and arachidonic of rat liver were not significantly affected by the dietary treatment. However, the linoleic content of rat fed winged bean yolk was higher ($p<0.05$) than of rats fed animal tallow yolk. On the other hand, rats fed winged bean yolk had a lower ($p<0.05$) linoleic content than did rats fed the soybean or corn yolk. These results were in agreement with the yolk fatty acid data (table 2). Winged bean yolk had a higher linoleic acid content than did animal tallow yolk. However, the winged bean yolk had a lower linoleic content than did soybean or corn yolk. As reported by other researchers, the fatty acid composition of liver lipid was significantly influenced by the fatty acid composition of the diet (Vilchez et al. 1990, 1991; Jiang and Sim, 1992).

The linolenic acid content of rats liver fed winged bean yolk was lower ($p<0.05$) than that of rats fed the animal tallow yolk, even though the linolenic acid content in winged bean yolk was higher. These results show that the fatty acid composition of liver lipid does not always reflect the fatty acid composition of the dietary fat.

These findings were in agreement with those of Hood (1990) who reported that quails fed with tuna oil, which rich in n-3 fatty acid, showed a marked reduction in n-3 fatty acid and increased in saturated fatty acid in the liver.

In conclusion, winged bean yolk seems to have adverse effects on rats as indicated by a lower body weight gain, lower levels of favorable cholesterol (HDL) and higher levels of unfavorable cholesterol (LDL).

**Table 6.** Effects of dietary treatments on liver fatty acids composition of rats (mg/dl)

| Fatty acids (%) | Treatments[1] | | | | SEM |
|---|---|---|---|---|---|
| | A | B | C | D | |
| Myristic | 0.49 | 0.58 | 0.57 | 0.53 | 0.05 |
| Palmitic | 21.94 | 21.59 | 22.10 | 22.20 | 0.43 |
| Palmitoleic | 6.71 | 5.99 | 5.51 | 6.42 | 0.49 |
| Stearic | 25.40 | 26.16 | 26.04 | 24.44 | 1.17 |
| Oleic | 31.66[a] | 28.75[b] | 30.44[ab] | 31.89[a] | 0.98 |
| Linoleic | 7.74[c] | 11.14[a] | 10.65[a] | 9.21[b] | 0.39 |
| Linolenic | 2.31[a] | 1.45[b] | 0.77[b] | 1.34[b] | 0.27 |
| Arachidonic | 4.07 | 4.35 | 3.91 | 3.98 | 0.62 |

[a,b,c] Means (n=7) in same row with different superscript differ significantly ($p<0.05$).

[1] A=basal diet+15% tallow yolk, B=basal diet+15% soybean yolk, C=basal diet+15% corn yolk, D=basal diet+15% winged bean yolk.

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