Effects of Feeding Winged Bean Oil on Cholesterol and Lipid Contents in Egg and Liver, and Fatty Acid Composition of Egg in Japanese Quail

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ABSTRACT : The purpose of this experiment was to study the effect of feeding winged bean (*Psophocarpus tetragonolobus*) oil on egg cholesterol and fatty acid of Japanese quail . Four groups of 10-week-old Japanese quails (n=10 per group) were fed a basal diet (20% CP, 2890 kcal/kg) supplemented with 5% of either animal tallow, soybean oil, corn oil or winged bean oil over an 8-week period. At the end of the experiment, 7 birds in each treatment were killed by decapitation. Blood samples and livers were collected for cholesterol analyses. There were no significant differences in final body weight, feed intake, egg production, egg and yolk weight due to the different oil treatments. Egg and plasma cholesterol levels obtained with the winged bean oil diet were higher than those obtained with the animal tallow or soybean oil diets, but were not significantly different from those obtained with corn oil diet. Egg lipid, liver lipid and liver cholesterol content was lower with the winged bean oil diet than with the animal tallow diet. It may be concluded that the winged bean oil diet did not impair laying performance, but increased egg and plasma cholesterol levels compared with soybean oil or animal tallow diets. Winged bean oil diet produced eggs with a higher oleic content and lower linoleic content compared with the soybean or corn oil diet. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 3 : 376-380*)

Key Words : Winged Bean Oil, Japanese Quail, Egg Cholesterol, Egg Fatty Acid

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 altitudes (NAS, 1981). The chemical composition of the mature seeds is very similar to that of soybean, and therefore offers the same uses as soybean. 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). Both the amino acid composition and the amino acid digestibility of winged bean protein are similar to those of soybean protein (Okezie and Martin, 1980; Mutia and Uchida, 1992, 1993; Wyckoff et al., 1983). Most of the research has focussed on winged bean protein. However, little information is available on winged bean oil and fatty acids.

The fatty acid composition of winged bean is similar to that peanut oil. Total unsaturated fatty acids account for 65% of the total fatty acids, and the ratio of unsaturated to saturated is about 2 (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 tocopherols. 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 seems unlikely to have other ill effects (Cerney et al., 1971; Khor et al., 1982; NAS, 1981).

Recently, the fatty acid and cholesterol contents of chicken egg have received increased attention due to the relationship between dietary lipid and the incidence of coronary heart disease (Shafey and Cham, 1994). There have been numerous reports on general effects of including animal and vegetable fats in poultry diets. It is well known that dietary intake of n-6 polyunsaturated fatty acids, especially linoleic acid which is abundant in vegetable oil, is effective in lowering blood cholesterol level compared with saturated fat (animal fat). Winged bean is one of the tropical crops that has a high oil content comparable to that of the soybean, but its potency remains unstudied.

The purpose of this study was to investigate the effect of feeding winged bean oil on the cholesterol content and fatty acid composition of Japanese quail eggs. For this purpose, three other lipids were used as comparison.

MATERIALS AND METHODS

Diets and animals

The winged bean seeds were obtained from cultivars locally grown in Indonesia. Seeds were milled, and then the oil was extracted with ether in a Soxhlet apparatus for 6 h. Four diets were prepared by mixing 5% of either animal tallow, soybean oil, corn oil (purchased from commercial sources) or winged bean oil with a formulated basal diet that meets NRC (1994) requirements. The composition of the basal diet is shown in table 1 and the fatty acid compositions of the experimental diets are presented in table 2. To prevent lipid peroxidation, the diets were mixed every week and stored in sealed containers at 4℃.

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 14L:

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10D h of light-dark cycle. Birds were fed fresh daily. Feed and water were available for *ad libitum* intake.

Table 1. Composition of basal diet

Ingredients	Amounts		
Corn	39.8	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 per kg diet contained: $CaHPO_4$, 1.75%; NaCl, 0.252%; $K_3C_6H_5O_7 \cdot H_2O$, 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% ZnO), 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 per kg diet contained: Thiamine, 6 mg; Riboflavin, 6 mg; Piridoxin, 7 mg; Nicotinic acid, 30 mg; D-Pantothenic 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.

Table 2. Fatty acid composition of experimental diet

Fatty acids (%)	Treatment[1]			
	A	B	C	D
Myristic	2.65	0.24	0.23	0.28
Palmitic	19.34	14.84	12.95	15.72
Palmitoleic	2.99	0.96	1.08	1.20
Stearic	5.85	4.02	3.51	3.58
Oleic	27.05	16.16	20.57	23.21
Linoleic	34.21	50.19	46.99	43.89
Linolenic	5.02	7.65	5.50	5.13
Behenic	0.55	-	-	-

The values presented are means of triplicate 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 oil.

Sample collection and analyses

Eggs were collected daily and egg production was recorded. Feed intake was measured weekly. After 8 wk on the experimental diets, two yolks from each bird were weighed, pooled and a sample was extracted using chloroform:methanol (2:1, vol/vol) according to the method of Folch et al. (1957). Yolk total lipid was determined gravimetrically. Methyl esters of the lipid extract were prepared according to Morrison and Smith (1974). Fatty acid composition of yolk lipids 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 temperature 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 egg samples was extracted by the method of Folch (1957). The samples were saponified by the method of Abell et al. (1952) modified by Beyer and Jensen (1989). The concentration of cholesterol was determined enzymatically using a commercial kit (Sterozyme-545) according to the manufacturer's instructions.

At the end of an experimental day, 7 birds from each treatment were weighed and killed by decapitation. Blood samples were collected into heparinized tubes and centrifuged at 4℃ at 3,000 rpm for 15 min to separate the plasma. Plasma cholesterol was analyzed as described above. The livers were immediately removed and weighed and then stored at -20℃ for further analyses. For liver lipid and cholesterol, the same procedures were applied as for the egg analyses.

Statistical analyses

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

RESULTS AND DISCUSSION

Fatty acid composition of winged bean oil

The fatty acid composition of winged bean oil used in this experiment was compared to the composition reported by other workers (table 3). Khor et al. (1982) investigated 30 varieties of winged bean seeds (15 varieties from New Guinea and 15 varieties from Thailand).

Table 3. Fatty acids of winged bean oil

Fatty acids (%)	Our results[1]	Khor et al. (1982)
Myristic	0.14	0.1-0.2
Palmitic	12.82	4.7-10.2
Palmitoleic	0.45	0.1-0.5
Stearic	5.33	4.2-8.4
Oleic	34.68	29.6-39.3
Linoleic	29.39	24.1-34.2
Linolenic	1.61	1.0-2.0
Arachidic	3.26	0.4-1.9
Behenic	12.32	14.2-17.4

[1] The value presented are means of triplicate determinations.

Our results are generally in agreement with those of Khor et al. (1982). However, palmitic and arachidic content of winged bean oil in this experiment were higher than those of Khor et al. (1982). On the other hand, behenic acid contents were lower. Cerney (1971) and NAS (1981) also reported lower behenic acid content in their winged bean oil. The different contents of fatty acid may be explained by differences in varieties of winged bean used and differences in environments in which they were grown. The winged bean seeds used in this experiment were from a cultivar grown in Indonesia.

The total saturated and unsaturated fatty acid contents of winged bean oil in this experiment were 33.87% and 66.13%, respectively. These results were in agreement with those of other workers (Cerney et al., 1971; Khor et al., 1982; NAS, 1981).

Effects of winged bean oil on laying performance

There were no significant differences ($p>0.05$) for egg production, egg weight, and yolk weight among the treatments (table 4). These results demonstrate that feeding winged bean oil did not affect the laying performance. Furthermore, winged bean oil did not affect feed intake as compared with other oil treatments. These findings indicated that there is no unpleasant taste or any anti-nutritional factor that could suppress the feed intake. As reported by other researchers, winged bean seeds contain several anti-nutritional factors such as trypsin and chymotrypsin inhibitors, amylase inhibitors, phytohemagglutinins, tannins and perhaps saponin (de Lumen and Salamat, 1980; Kadam et al., 1987; Sathe and Salunkhe, 1981).

Table 4. Effects of oil treatments on laying performance

Response variable	Treatment[1]				SEM
	A	B	C	D	
Initial body weight (g)	131.22	130.31	130.80	129.46	2.92
Final body weight (g)	144.06	143.81	142.79	135.04	3.24
Egg production (%)	80.73	85.18	83.57	82.51	0.39
Egg weight (g)	9.88	9.60	10.03	9.63	0.24
Yolk weight (g)	3.22	3.11	3.26	3.09	0.07
Feed intake (g/day)	26.06	26.03	26.11	25.09	0.65

[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.

The final body weight of birds fed winged bean oil diet did not differ significantly ($p>0.05$) from those for the other oil treatments. Although there have been no reports on the effects of winged bean oil on laying performance, in a study of the growth rate of quails, Wyckoff et al. (1983) reported that quails given a winged bean meal diet supplemented with 0.5% methionine grew as well as on the soybean meal control diet. de Lumen et al. (1982) reported that replacement of as much as 95% of soybean meal with winged bean meal did not affect broiler performance. Our results show that feeding winged bean oil did not impair laying performance.

Effects of winged bean oil on lipid and cholesterol content

Table 5 summarizes the effect of feeding winged bean oil on lipid and cholesterol contents in egg and liver. Birds fed the winged bean oil diet had a significantly ($p<0.05$) lower egg lipid than those of birds fed animal tallow or corn oil diet. However, egg cholesterol with the winged bean oil treatment was significantly ($p<0.05$) higher than with animal tallow or soybean oil treatment. There are numerous reports on dietary factors and egg cholesterol. These range from alterations in nutrient content to the inclusion of feed ingredients and drugs that reduce cholesterol (Naber, 1976; Stadelman and Pratt, 1989; Luhman et al., 1990). However, experimental evidence on the effect of diet on egg cholesterol is equivocal and varies with the nature of the diet. Vilchez et al. (1991) reported that egg lipid and egg cholesterol were not significantly affected by dietary treatment. Although other workers have reported that dietary fat has little effect on cholesterol deposition in the egg yolk, the results in this experiment show that lipid and cholesterol contents in egg yolk were significantly ($p<0.05$) affected by dietary treatment.

Table 5. Effects of oil treatments on egg, plasma and liver cholesterol, liver weight, egg and liver lipid

Response variable	Treatment[1]				SEM
	A	B	C	D	
Egg lipid (%)	43.17[a]	40.84[bc]	42.28[ab]	40.49[c]	0.61
Egg cholesterol (mg/g yolk)	21.76[b]	19.73[c]	22.98[ab]	23.61[a]	0.49
Serum cholesterol (mg/dl)	121.30[c]	118.50[c]	167.40[ab]	176.40[a]	17.52
Liver weight (g/100 BW)	3.04	2.94	2.75	2.88	0.19
Liver lipid (%)	16.46[a]	13.37[ab]	14.17[ab]	9.60[b]	2.11
Liver cholesterol (mg/g)	7.64[a]	5.47[b]	6.87[ab]	5.63[b]	0.62

[a-c] Mean in same row with different superscripts differ significantly ($p<0.05$).

[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.

In agreement with the egg cholesterol data, the birds that were fed winged bean oil diet had a significantly ($p<0.05$) higher serum cholesterol than those birds fed animal tallow or soybean oil diet. Although other reports have not shown consistent results, our results showed that birds with higher serum cholesterol produced eggs with higher cholesterol content. Because yolk cholesterol is derived from plasma cholesterol, it is logical to assume that a relationship should exist between yolk and plasma cholesterol. However, such a relationship is not clear. Sim and Bragg (1977) reported that changes in egg yolk cholesterol level were closely related to changes in serum cholesterol level. However, Weiss and Scott (1979) reported that the ovarian synthesis of cholesterol is responsible for maintaining the level of cholesterol in the egg, and that plasma cholesterol levels have little effect on egg cholesterol levels. In this experiment, egg and serum cholesterol levels of eggs fed the winged bean oil diets were higher than those obtained with the animal tallow or soybean oil diet, but not different from those obtained with the corn oil diet.

Liver weight was not significantly ($p>0.05$) affected by the different oil treatments (table 5). Liver lipid and cholesterol of birds fed winged bean oil diet were significantly ($p<0.05$) lower than those of birds fed tallow but were not significantly ($p>0.05$) different from those obtained with the soybean or corn oil diet. This indicated that winged bean oil does not result in a fatty liver.

Effects of winged bean oil on yolk fatty acid

The effects of feeding winged bean oil on yolk fatty acid are summarized in table 6. The birds fed the diet with animal fat (tallow) produced eggs whose yolk had significantly ($p<0.05$) higher myristic and oleic content and significantly ($p<0.05$) lower stearic and linoleic contents than yolk of birds fed the diet with plant oils. These results were in agreement with fatty acids in the diet (table 2). However, the animal fat diet had a lower linoleic content than did the plant oil diets. Our results indicated that egg yolk fatty acid reflected the fatty acid composition in the diet. These findings are in agreement with those of Vilchez et al. (1990, 1991) who reported that fatty acids of egg yolk, plasma and liver lipids were significantly influenced by the fatty acids of the diet. It is interesting to note that the palmitic content in egg yolk did not significantly ($p>0.05$) differ among the treatments, even though the palmitic contents in the diets were different. The results of this experiment show that the palmitic content in egg yolk was not affected by the diet. A possible explanation may be that the deposition of palmitic is required and is achieved by *de novo* synthesis regardless of its amount in the diet.

Birds fed a plant oil diet had higher ($p<0.05$) levels of saturated fatty acids (SFA) and poly-unsaturated fatty acid (PUFA), but lower levels of mono-unsaturated fatty acid (MUFA) in their yolk as compared with the birds fed the animal fat diet. These results correspond to the levels of fatty acid in the diet. The plant oil diets had higher PUFA than did the animal tallow diet. Sim et al. (1973) reported that feeding diets containing high levels of poly-unsaturated fatty acids resulted in decreased synthesis of mono-unsaturated fatty acids and increased synthesis of saturated fatty acids. Vilchez et al. (1991) also reported that the synthesis of mono-unsaturated fatty acids is considerably reduced, whereas the synthesis of saturated fatty acids is increased in order to maintain a specific saturated to unsaturated ratio during a period of poly-unsaturated ingestion. Furthermore, Naber (1979) reported in a review that almost all of the change in the contents of linoleic and linolenic acids is accomplished by substitution for oleic acid in the yolk lipid. Under conditions of minimum dietary fat intake, the predominant fatty acid synthesized for incorporation into yolk lipid is oleic acid. Under a wide variety of feeding conditions, the sum of the contents of the three 18-carbon fatty acids tends to be approximately constant. In this study, the sums of three 18-carbon fatty acids (oleic, linoleic and linolenic) were 49.12, 47.14, 47.78, and 48.24% for the animal tallow, soybean, corn and winged bean oil diets, respectively.

Table 6. Effects of oil treatments on egg fatty acid

Fatty acids (%)	Treatment[1]				SEM
	A	B	C	D	
Myristic	0.37^{a}	0.25^{b}	0.18^{b}	0.24^{b}	0.02
Palmitic	30.61	31.76	30.85	30.44	0.69
Palmitoleic	2.99^{a}	1.87^{b}	2.30^{ab}	2.35^{ab}	0.26
Stearic	15.63^{b}	18.27^{a}	17.61^{a}	17.80^{a}	0.52
Oleic	37.65^{a}	24.81^{d}	26.92^{c}	32.45^{b}	0.52
Linoleic	10.89^{c}	21.68^{a}	20.30^{a}	15.35^{b}	0.56
Linolenic	0.58	0.65	0.56	0.44	0.13
Arachidonic	1.28^{a}	0.72^{b}	1.28^{a}	0.93^{ab}	0.16
SFA[2]	46.61^{c}	50.27^{a}	48.64^{b}	48.48^{b}	0.62
MUFA[3]	40.64^{a}	26.68^{d}	29.22	34.80^{b}	0.47
PUFA[4]	12.75^{c}	23.05^{a}	22.14^{a}	16.17^{b}	0.63

[a-d] Mean in same row with different superscripts differ significantly ($p<0.05$).
[1] A=basal diet+5% tallow; B=basal diet+5% soybean oil; C=basal diet+5% corn oil; D=basal diet+5% winged bean oil.
[2] SFA (saturated fatty acid)=Myristic+Palmitic+Stearic.
[3] MUFA (mono-unsaturated fatty acid)=Palmitoleic+Oleic.
[4] PUFA (poly-unsaturated fatty acid)=Linoleic+Linolenic+Arachidonic.

When comparing the results among the plant oil treatments, feeding winged bean oil produced yolk with a higher ($p<0.05$) oleic content than soybean or corn oil. However, the yolk linoleic content in the winged bean oil treatment was significantly ($p<0.05$) lower than other plant oil treatments. Therefore, birds fed the winged bean oil diet had significantly ($p<0.05$) higher MUFA but significantly ($p<0.05$) lower PUFA than did the birds fed soybean or corn oil diet. These results were in agreement with the levels of fatty acid in the diet. The winged bean oil diet had higher oleic but lower linoleic content than did the soybean or corn oil diet.

In conclusion, a winged bean oil diet for Japanese quail did not impair laying performance, but increased egg and plasma cholesterol levels compared with soybean oil or animal tallow diets. When winged bean oil was supplied, egg lipid, liver lipid and liver cholesterol decreased compared to animal tallow and the content of egg oleic was higher but linoleic lower as compared with soybean or corn oil diets.

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