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Fatty Acid Composition of Yolk Lipids of Eggs from Ducks Fed the Natural and Formula Feeds

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Introduction

Differing from the chicken and the quail belonging to the Galliformes, the duck (*Anas platyrhynchos*) is a kind of waterfowl belonging to the Anseriformes. Generally it is reared on or near water of the river or pond, usually ingesting the natural diets such as small fishes and water grasses in the river or pond; sometimes it feeds the residues of human diet. It is said that in the People's Republic of China, duck eggs as a human diet have been preferred to hen eggs. In this country, Pidan prepared from the duck egg is frequently set as one of the fixed dishes on a Chinese meal. The egg is a rich source of lipid as well as the hen egg, because of its being rather richer in lipid than in case of the latter.[6]

The fatty acid composition of yolk lipid of the hen egg is influenced by the type of lipid in the diet. The past research has shown that total amount of saturated fatty acids does not change even with a large alteration in dietary fatty acid composition, but that the linoleic acid content of yolk increases with a concurrent decrease in oleic acid when the level of dietary polyunsaturated fatty acids is raised.[1,2,3,8,10]

There is, however, few research concerning the influence of diet on the fatty acid composition of the duck yolk lipid. The present paper describes the difference between the fatty acid compositions of yolk lipids of eggs laid by ducks fed the natural and formula feeds.

Materials and Methods

Experimental materials — Eggs laid by ducks fed the natural diet at the outfall of the river, Nagata in Kagoshima City and those by ducks fed the commercial formula feed for the laying hen at our laboratory were used as the experimental materials.

The commercial formula feed was purchased from Toyohashi Feed Joint - Stock Company. The material combination and the general constituent were shown in Table 1 and 2. Egg yolks were separated from their respective whites. Traces of egg white which adhered to the yolk were carefully removed with the aid of injector, adding a small amount of water. The yolk was blended with a homogenizer at a slow speed, lyophilized and stored in a sealed polyethylene vessel introduced nitrogen gas, at $-15°C$.

Extraction of yolk lipids — Total lipids were extracted with chloroform-methanol (2:1, *v/v*) mixture at room temperature according to the method of Folch *et al.*[4] To 20 g of the dried yolk, 3 volume of the above extractant was added and it was allowed to stand for 30 min; homogenized

Table 1. Material combination of the commercial formula feed for laying hen

Materials	Ratio (%)	Detail of materials
Grain	63	corn, unhulled-rice
Plant seed meal	15	soybean meal, corn gluten meal, rape seed meal
Dregs and bran	7	corn gluten feed, rice bran, screening pellet
Animal feed	6	fish meal, fish soluble adsorption feed, meat bone meal, feather meal
Others	9	molasses, $CaCO_3$, $Ca_3(PO_4)_2$, NaCl, canthaxanthin, black locust leaf meal

Materials and their combination ratio have been published from Toyohashi Feed Joint-Stock Company.

Table 2. General chemical composition of the commercial formula feed

Component	Crude protein	Crude fat	Crude fibre	Crude ash
Content (%)	> 17.0	> 3.0	< 6.0	< 13.0
Compnent	Ca	P	ME*	
Content (%)	> 2.5	> 0.55	> 2750 Kcal/Kg	

* Metabolizable energy

Values have been published from Toyohashi Feed Joint-Stock Company.

for 5 min and filtered. After repeating the same operation 4 times, total extracting solution was transferred into a separatory funnel and 80 ml of water was added, shaken adequately. After allowing to stand for a few minutes, the under chloroform layer was poured into another vessel and sodium sulfate anhydride powder was added, and then left to stand overnight, filtered, concentrated under reduced pressure, finally adjusted to the 100 ml-volume with chloroform.

Fractionation of total yolk lipids — Total lipids were fractionated into the neutral glyceride and polar phospholipid fractions by the silicate column chromatography. The column employed was a size of dia. 2.4 cm, length 32 cm, filled with Wakogel C-100 activated previously by heating at 100°C overnight. Preliminary experiment showed that the flow of organic solvent through the Wakogel C-100 column is faster than that through the gel C-200 column, accompanied with a good separation of the fractions of the lipid as will be described later.

On chromatography, the column was firstly rinsed with 100 ml of chloroform. Ten ml of the lipid sample in chloroform solution was applied on the silicate column, and the neutral glyceride and polar fractions were eluted with chloroform-methanol (20:1, *v/v*) followed with anhydrous methanol.

The respective fractions separated were evaporated in a rotatory evaporator in a water bath at 40°C and the residues weighed.

Thin layer chromatography of the neutral glyceride and polar fractions — To ascertain a good separation of the two fractions, the thin layer chromatography was conducted. The conditions employed were as follows; The 20 × 20 cm size of the Wako silica gel-60 thin layer plate (activated at 110°C overnight), the ascending method using petroleum ether-ethylether-acetic acid (80:30:1) and chloroform-methanol-water (65:25:4) as the developing solvent. Two plates on which the same sample was developed were used for the development of color after being sprayed with 50 % sulfuric acid (for the neutral glyceride) or molybdenum blue reagent (for the phospholipid),

followed by heating at 100°C.

Preparation of methyl esters — The preparation of fatty acid methyl esters from samples by interesterification was achieved in accordance with the procedure described by Takahashi *et al.*,[12] referring to the method of Stoffel *et al.*[11] at the time. Total lipids, neutral glyceride and phospholipid containing 5 ~ 10 mg as fatty acids were separately used as samples.

The sample was dissolved in 8 ml of anhydrous methanol saturated with hydrogen chloride and 1 ml of benzene, and the mixture was refluxed, with frequent shakings, in a water bath at 80 ~ 100°C until only one phase was observed (approximately 2 ~ 3 hr). After cooling to room temperature, 3.5 ml of water was added, the methyl esters were extracted three times with 20 ml of petroleum ether. The pooled extracts were concentrated to a small volume under reduced pressure and diluted to 25 ml with petroleum ether. The solution was then simultaneously neutralized and dehydrated with sodium sulfate anhydride and sodium bicarbonate (4:1, *w*/*w*) and the supernatant was used as the sample for gas-liquid chromatography.

Gas chromatography — The methyl esters were analyzed in a Shimazu GC-4B gas chromatograph equipped with a hydrogen flame ionization detector. The conditions employed were as follows: stainless column 3 mm × 3 m; packings, DEGS-H_3PO_4 (5 %, 1 %); support, Chromosorb W 60 ~ 80 mesh; column temperature 200°C; detector temperature 235°C; injection temperature 220°C; carrier gas, nitrogen gas with a flow rate 50 ml/min.

Chromatographic peaks were identified by comparing their retention times with those of known standards. Quantitation of the various peaks was accomplished by triangulation.

Results and Discussion

The ratio of the neutral glyceride to polar lipid fractions separated by the silicate column chromatography was 94:6 in the yolk lipid of the duck egg on the natural diet and 96:4 in that on the formula feed, differing remarkably from the ratio 72:28 for the hen yolk lipid obtained by Fujino (1971)[5]. As shown in Fig. 1, on the thin layer chromatogram with the petroleum ether-ethylether-acetic acid solvent, the color developing with sulfuric acid revealed three spots in the neutral glyceride fraction and no spot in the polar fraction, while the color developing with molybdenum blue revealed only one spot staying at the start line in the phospholipid fraction and no spot in the glyceride fraction. On the chromatogram with the chloroform-methanol-water solvent in Fig. 2, the absence of phospholipid in the neutral glyceride fraction and the absence of neutral glyceride in the polar fraction were well ascertained likewise in Fig. 1. These results show that the separation of total lipid into the neutral glyceride and phospholipid fractions to be necessary for the present investigation is satisfactory, even if further separation of the two fractions to their constituents is inadequate.

From the preceding descriptions, the remarkably small proportion of the phospholipid fraction was noticeable, but the reason was obscure. Fatty acid compositions of the yolk lipids in eggs laid by ducks on the natural and formula feeds were shown in Table 3, accompanied with the composition of yolk lipid in eggs of hens on the same formula feed. Stearic and oleic acids contents of the duck yolk lipid were larger in the natural diet group than in the formula feed group, while the other acids contents were smaller in the former than in the latter. The ratio of unsaturated to saturated acids in the natural diet group was higher than that in the formula feed group.

The comparison between the fatty acid composition of yolk lipid in eggs of the duck and that of the hen, fed the same feed, showed that oleic and palmitic acids were major constituents in the

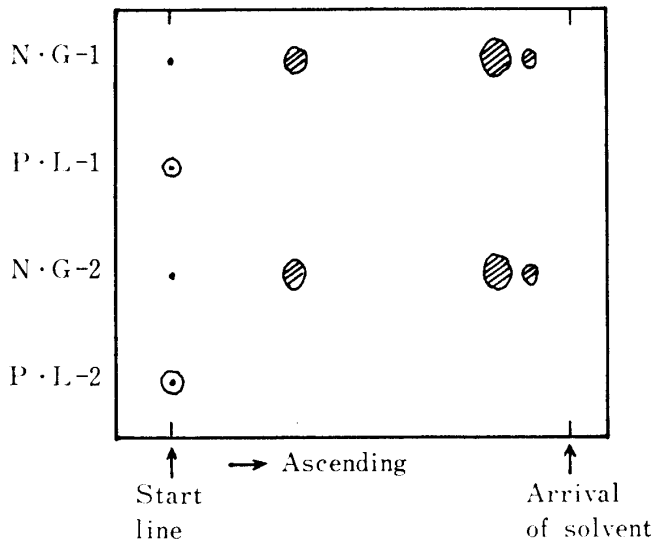

Fig. 1. Thin layer chromatogram of the neutral glyceride and phospholipid fractions of duck yolk lipids.
Developing solvent: petroleum ether-ethylether-acetic A. (80:30:1)
Coloring reagent: 50% H_2SO_4 for detecting neutral glyceride, molybdenum blue reagent for detecting phospholipid
N·G-1: neutral glyceride fraction (from egg yolk on the natural diet group)
P·L-1: phospholipid fraction (〃 〃 〃)
N·G-2: neutral glyceride fraction (from egg yolk on the formula feed group)
P·L-2: phospholipid fraction (〃 〃 〃)

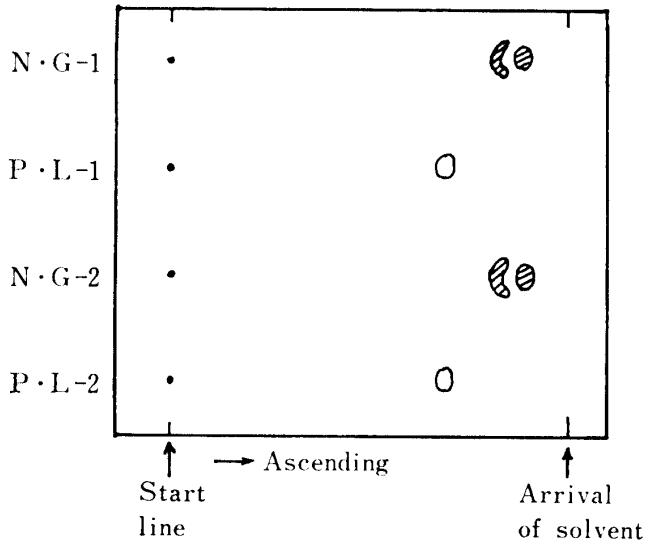

Fig. 2. Thin layer chromatogram of the neutral glyceride and phospholipid fractions of duck yolk lipids.
Developing solvent: chloroform-methanol-water (65:25:4)
Coloring reagent: 50% H_2SO_4 for detecting neutral glyceride, molybdenum blue reagent for detecting phospholipid
N·G-1, P·L-1, N·G-2, P·L-2: the same as the explanation in Fig. 1.

Table. 3. Fatty acid composition of yolk lipids of eggs from ducks fed the natural and formula feeds

Fatty acid	Duck yolk lipid, natural feed	Duck yolk lipid, formula feed	Hen yolk lipid,* formula feed
14:0	0.4	0.6	0.4
14:1	—	—	0.4
16:0	29.2	33.4	26.3
16:1	4.4	4.7	3.4
18:0	7.0	5.3	10.7
18:1	49.7	46.1	48.3
18:2	6.2	6.6	10.0
21:0	—	—	0.5
20:4	3.1	3.3	—
Satd. acid (A)	36.6	39.3	37.9
Unsatd. acid (B)	63.4	60.7	62.1
B/A	1.73	1.54	1.64

Values were expressed as percentage of total fatty acids.
* Analytical values were previously reported by authors. Reference No. (7)

duck yolk lipid as well as in the hen yolk lipid, and that stearic, oleic and linoleic acids contents in the duck lipid were lower than those in the hen lipid, while myristic, palmitic and palmitoleic acids in the duck were higher. Although Ootake *et al.*[9] have shown the content of 1.5 % arachidonic acid as percentage of total acids in the hen yolk lipid, we could not detect the presence of the acid in hen yolk lipids.

Chen *et al.*[1] showed the absence of this acid in hen yolk lipids. In the present study, about twofold amount of the arachidonic acid content obtained by Ootake *et al.*[9] in the hen yolk lipid was found in the duck yolk lipid. Myristoleic and heneicosanoic acids could not be detected in the duck yolk lipid. The ratio of unsaturated to saturated acids in duck lipid was slightly smaller than that in the hen lipid. The fatty acid composition of the hen yolk lipid obtained by us was quite similar to the analytical result of Chen *et al.*[1] on the hen yolk lipid.

The fatty acid compositions of the neutral glyceride and phospholipid fractions were shown in Table 4 and 5. Six kinds of acids were detected in the duck yolk glyceride fraction, coinciding with the analytical data of Chen *et al.*[1] on the hen yolk glyceride. The composition is similar to that of the total lipids of the duck yolk. This similarity may be ascribed to the extremely large proportion of the neutral glyceride in the total lipids. The ratio of unsaturated to saturated acids was higher in the natural diet group than in the formula feed group, and moreover, the ratios in these two groups were slightly larger than the respective ratios in the total lipids shown in Table 3. Especially, the predominance of oleic acid content in the neutral glyceride of the natural diet group was noteworthy.

The ratios of unsaturated to saturated acids in the phospholipid fractions of egg yolks from two sorts of diet groups were almost the same, being much smaller than 1.0 (Table 5) and markedly different from their respective ratios in the neutral glyceride fractions. However, comparing the fatty acid composition of the phospholipid in the natural diet group with that in the formula feed group, stearic and oleic acids were slightly larger in the former than in the latter in quantity, while palmitic and linoleic acids were respectively smaller. Although it was proper that arachidonic acid should be detected, authors failed in the detection. In the experiment of Chen *et al.*[1], the

Table 4. Fatty acid composition of the neutral glyceride fraction of duck egg yolk

Fatty acid	Natural feed	Formula feed
14:0	0.6	0.6
16:0	27.3	30.8
16:1	4.3	5.2
18:0	5.4	4.5
18:1	56.8	52.5
18:2	5.6	6.4
Satd. acid (A)	33.3	35.9
Unsatd. acid (B)	66.7	64.1
B/A	2.00	1.78

Values were expressed as percentage of total fatty acids.

Table 5. Fatty acid composition of the phospholipid fraction of duck egg yolk

Fatty acid	Natural feed	Formula feed
16:0	43.7	45.2
16:1	trace	trace
18:0	18.5	17.2
18:1	30.1	29.4
18:2	7.7	8.2
Satd. acid (A)	62.2	62.4
Unsatd. acid (B)	37.8	37.6
B/A	0.61	0.60

Values were expressed as percentage of total fatty acids.

increase of stearic acid, the decrease of oleic acid and the uncertain change of palmitic acid in the total lipids of hen yolk were observed when such plant seed oils as linseed oil and cotton seed oil were added on the control diet by 10 %. On the contrary, in our experiment, the increase of oleic acid except stearic acid and the decrease of palmitic acid in the total lipids of duck yolk on the natural diet group in comparison with those acids on the formula feed group, were observed.

Since the precise constituents of the natural diet ingested by ducks were obscure, the conclusion regarding the relationship between the fatty acid compositions of duck yolk lipid and diet lipid could not be obtained.

However, authors emphasize that the difference of the feeds for ducks influences chiefly the fatty acid composition of the neutral glyceride in the egg yolk.

Summary

1. The comparison between fatty acid composition of yolk lipids of eggs laid by ducks fed the natural diet and that by ducks fed the formula feeds gave an ascertainment that the contents of stearic and oleic acids in the total lipids were larger in the natural diet group than in the formula feed group, while palmitic, palmitoleic, linoleic and arachidonic acids were smaller in the former than in the latter, and the ratio of unsaturated to saturated acids in the natural diet group was higher than that in the formula feed group. Oleic and palmitic acids were major constituents in the duck yolk lipid as well as in the hen yolk lipid, however, stearic, oleic and linoleic acids contents of the duck yolk lipid were smaller than those of the hen yolk lipid, while myristic, palmitic, palmitoleic and arachidonic acids contents in the duck yolk lipid were larger.

2. Total yolk lipids of the duck egg were separated into the neutral glyceride and phospholipid fractions by the silicate column chromatography.

The separation was ascertained to be qualitatively adequate by the thin layer chromatography. The fatty acid compositions of the neutral glyceride fractions in the natural and formula feed groups were approximately similar to those of total lipids. The ratios of unsaturated to saturated acids in the two feed groups were slightly larger than the respective ratios in the total lipids. The ratios of unsaturated to saturated acids in the phospholipid fractions in the two feed groups were almost the same, being much smaller than 1.0 and remarkably different from the respective ratios in the neutral glyceride fractions.

3. From these results, it was presumed that the feeds for ducks give influences chiefly on to the fatty acid composition of the neutral glycerides in the egg yolk, while they scarcely do on to the composition of the phospholipids.

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