Composition and Structure of Triacylglycerol of Muscle Lipids from Laying Hen as Influenced by Dietary Fats

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The effects of dietary fats (soybean oil, coconut oil, lard and beef tallow) on the changes in composition and structure of triacylglycerol (TG) in muscle lipids of laying hens were studied. The fatty acid composition found in muscle total lipids from each dietary treatment group reflected the composition of fats added to the rations. The TG from thigh and breast muscle were subjected to stereospecific analysis. Generally, C_{16:0}, C_{16:1}, and C_{18:0} were preferentially esterified at positions sn-1 and sn-3. C_{18:1} tends to linked to position sn-3 more than other positions, and C_{18:2} was predominated in position sn-2 of muscle TG. TG species were resolved on the basis of carbon number by high temperature gas-liquid chromatography. The muscle lipids from soybean oil, lard and tallow groups commonly comprised the TG of C₅₀, C₅₂ and C₅₄ as the major TG components, and those from coconut oil group contained many medium chain length TG as C₃₄ ~ C₄₈ components in addition to C₅₀ ~ C₅₄. The TG was separated into simpler molecular species according to the degree of unsaturation by argentation thin-layer chromatography (AgNO₃-TLC). The lipids from soybean oil group contained less S₂M and SM₂ and more of M₂D, SD₂, MD₂, SMT and D₃ species than the other groups, while the lipids from coconut oil group had the highest level of S₃ and S₂M species among the dietary groups. The experimentally determined values of TG species were compared with the values predicted assuming a 1-random, 2-random, 3-random arrangement of fatty acids in intact TG. The agreement was considerably good for muscle TG from lard and tallow groups, but some discrepancies were observed for TG from soybean oil and coconut oil groups. This suggests that in laying hen there is preferential synthesis of some TG species influenced by ingested dietary fatty acids.

The fatty acid composition of tissues of chicken has been studied by a number of researchers¹⁾⁻⁵⁾, and considerable information has been reported concerning the influence of dietary fats on fatty acid composition of lipids in chicken tissues⁶⁾⁻¹¹⁾. The fatty acid composition of body fat in animals is generally a reflection of the dietary fatty acid pattern. The degree of unsaturation of fatty acids in chicken tissues is influenced by the degree of unsaturated fatty acids in diet and tends to assume the fatty acid composition of the diet⁷⁾. The nature, proportion, and degree of unsaturated fatty acids present in lipid will indicate the approximate susceptibility of lipid towards oxidative deterioration⁴⁾. The fatty acid com-

position of body fats is primarily influenced by dietary fatty acids and results from highly specific and controlled metabolic reactions which concern not only the influence of one fatty acid on the distribution of other fatty acids, but also the position of the fatty acids on the glycerol molecule¹²⁾. In addition to gross fatty acid composition, the distribution of fatty acids among three positions of L-glycerol is an important factor in determining the physical properties of muscle tissues TG¹³⁾. There have been many reports on the effects of different dietary treatments upon the gross fatty acid compositions of TG in chicken muscle tissue, but there is few information available on the effects of dietary fatty acids upon

the structure of tissue TG. This investigation is designed to elucidate the comparative effects of different dietary fats on the composition and molecular structure of muscle lipids from laying hens fed fat-supplemented diets.

Materials and Methods

Treatment of the birds and muscle samples

Twenty laying White Leghorn hens, 8 to 10 months of age, were divided into four groups of five each, and each group was given one of the experimental diet *ad libitum* for a period of 50 days. The composition of rations and fatty acid compositions of dietary fats described previously¹⁴⁾ are shown in Table 1.

After 50 days feeding on the experimental diets, hens were fasted overnight, sacrificed, and the thigh and breast muscles were removed

from carcasses for analysis. These muscle were freed of skin and visible fatty tissue and kept at -15°C until analyses were carried out.

Extraction of lipids and preparation of triacylglycerol

The muscle samples were minced by a home meat chopper with a plate of 3 mm pores. The total lipids were extracted from each minced meat sample according to the method of FOLCH *et al.*¹⁵⁾, and were separated into neutral and polar lipid fractions by the procedure of MOERCK and BALL¹⁶⁾. Then, TG was isolated from each neutral lipid fraction by Unicl (Clarkson Chem. Co.) silicic acid column chromatography as described by MOERCK and BALL¹⁶⁾.

Streospecific analysis

The fatty acid composition of each of three

Table 1 Composition of experimental diet and fatty acid compositions of supplemented fat or oil to the experimental diets

Ingredient	Content (wt, %)
Corn starch	34.549
Soybean oil meal	45
Fat (oil)	10
Salt (NaCl)	0.5
Calcium carbonate	6
Dicalcium phosphate	3.2
DL-Methionine	0.25
Vitamin premix*	0.3
Trace mineral premix**	0.2
Carophyll***	0.001

*Supplied amounts per kg of diet: Vitamin A, 15 000 I.U.; Vitamin D₂ 2 000 I.U.; Vitamin E, 20 I.U.; Vitamin K₃, 5 mg; Thiamin hydrochloride, 10 I.U.; Riboflavin, 10 mg; Calcium pantotheanate, 10 mg; Nicotinamide, 5 mg; Folic acid, 5 mg; Pyridoxine hydrochloride, 5 mg; Biotin, 0.5 mg; Vitamin B₁₂, 0.02 mg; Inositol, 100 mg; Calcium ascorbate, 250 mg; Cholin chloride, 1 000 mg

**Supplied amounts per kg of diet: MnCO₃, 150 mg; Fe-citrate, 100 mg; Na₂SeO₄·10H₂O, 0.5 mg; H₃BO₄, 1 mg; Zn-acetate, 50 mg; Na₂MoO₄·2H₂O, 5 mg; KIO₃, 0.5 mg; 2Co(OH₂)·H₂O, 0.5 mg

***Feed pigment (Roche)

	Soybean oil	Coconut oil	Lard	Tallow
Fatty acid*	(wt. %)			
8:0		7.3		
10:0		6.2		
12:0		47.3	tr	tr**
14:0	tr	18.9	1.5	3.1
14:1	tr		0.2	0.8
15:0	tr		0.1	0.4
16:0 Br				0.1
16:0	10.6	9.4	24.3	24.3
16:1			3.4	2.9
17:0				0.9
17:0 Br	0.1		0.4	1.2
17:1			0.3	0.6
18:0	3.8	2.8	12.5	18.8
18:1	22.5	6.5	44.7	42.1
18:2	54.1	1.5	10.7	3.7
18:3	8.6		0.7	0.5
20:1	0.3		1.1	0.7
Sat. acid	14.5	91.9	38.8	48.8
Unsat. acid	85.5	8.1	61.2	51.2

* Number of carbon atom : number of double bond, Br=branched chain acid

** tr=trace (bellow 0.1%)

positions of the sn-glycerol moiety was determined by a stereospecific analysis procedure based on that devised by BROCKERHOFF¹⁷⁾ and modified by CHRISTIE and MOORE¹⁸⁾. The scheme of analysis was briefly described previously¹⁹⁾.

Argentation thin-layer chromatography

Each TG sample was separated into simpler molecular species on the basis of total number and distribution of double bonds per molecule by argentation thin-layer chromatography (AgNO₃-TLC). Separation of TG was performed on AgNO₃ impregnated Silica Gel G plate prepared by the method of Wood and Snyder²⁰⁾, using hexane-diethyl ether-benzene-methanol (70 : 10 : 20 : 1, by volume) as the developing solvent²¹⁾. The amount of each TG fraction separated on chromatogram

was calculated with reference to the methyl heptadecanoate added as the internal standard, and the TG composition of those bands was calculated on the basis of their fatty acid composition, as described previously²²⁾.

Gas-liquid chromatography (GLC)

Direct gas-liquid chromatography of TG was carried out on Hitachi Model-163 Gas Chromatograph. The operating conditions of GLC and determining procedure of distribution of various TG types were as same as described previously¹⁹⁾. Distributions of various TG types by carbon number in muscle lipids were estimated by the method described by KUKSIS *et al.*²³⁾

The fatty acid composition of lipid was determined by the procedure described previously²⁴⁾, using Shimadzu GC-6 A MPE Gas Chro-

Table 2 Fatty acid composition of muscle total lipids of laying hens fed fat-supplemented diets (wt. %)

Fatty acid*	Thigh muscle				Breast muscle			
	SO**	CO**	L**	B**	SO**	CO**	L**	B**
10:0		0.2				0.3		
12:0	0.3	12.0	0.1	0.2	0.4	10.4	0.1	0.4
14:0	0.8	7.9	1.1	1.5	0.8	5.9	1.0	1.3
14:1	tr***	0.6	0.2	0.3	tr	0.3	tr	0.2
15:0	0.1	tr	0.1	0.2	tr	tr	tr	0.1
16:0	18.4	18.2	21.4	22.0	19.5	21.2	23.9	23.4
16:1	3.4	3.5	4.4	4.2	2.2	2.4	3.1	2.8
17:0	0.5	0.2	0.4	0.5	0.3	0.1	0.3	0.3
17:1	0.1			0.3			0.1	0.1
18:0	6.7	7.2	8.9	10.2	6.8	7.4	8.2	9.2
18:1	36.1	31.9	44.0	43.2	33.4	29.2	40.9	39.5
18:2	29.3	15.2	15.9	13.9	29.7	15.1	15.4	14.5
18:3	2.2	0.5	0.5	0.6	2.1	0.4	0.5	0.5
20:1	0.6	0.5	0.6	0.6	0.4	0.3	0.5	0.5
20:2	0.3	0.2	0.2	0.2	0.1		0.1	
20:3	tr	tr	tr	tr	0.1	0.2	0.2	0.2
20:4	1.0	1.6	1.6	1.8	2.9	4.6	3.9	4.9
22:4		tr	0.1			0.3	0.1	0.1
22:5		tr	0.1		0.2	0.3	0.3	0.4
22:6	0.2	0.2	0.3	0.2	1.0	1.5	1.3	1.4

* Number of carbon atoms : Number of double bonds

** SO=soybean oil, CO=coconut oil, L=lard and B=beef tallow feeding

*** tr=trace (below 0.1%)

matograph and Shimadzu Chromatopac C-R2A.

*composition of muscle lipids of laying hen***Results and Discussion***Effects of dietary fats on the fatty acid*

The fatty acid compositions of total lipids obtained from thigh and breast muscles of laying hens fed diets supplemented with 10% of

Table 3 Positional distribution of fatty acids in triacylglycerols of chicken muscle lipids from hens fed fat-supplemented diets (mole %)

Treatment	Position*	12:0	14:0	16:0	16:1	18:0	18:1	18:2	Others***	Sat. acid	Unsat. acid
Thigh muscle											
Soybean oil	TG**	0.4	1.0	19.8	3.5	5.9	37.9	28.2	3.3	27.6	72.4
	1	0.9	1.6	28.4	5.4	8.3	36.8	16.2	2.4	39.8	60.2
	2	0.4	1.0	12.9	2.3	3.2	34.8	41.9	3.5	17.9	82.0
	3		0.4	18.1	2.8	6.2	42.1	26.5	3.9	25.2	75.0
Coconut oil	TG	18.5	9.8	17.9	3.9	4.8	29.6	12.4	3.1	52.1	47.9
	1	16.2	10.8	21.3	4.8	6.6	27.8	10.6	1.9	55.4	44.6
	2	14.0	9.1	13.9	2.9	3.2	31.1	23.2	2.6	40.6	59.4
	3	25.3	9.5	18.5	4.0	4.6	29.9	3.4	4.8	60.2	39.8
Lard	TG	0.3	1.4	23.3	5.2	6.7	45.9	15.0	2.2	32.2	67.8
	1	0.4	1.4	24.4	6.3	8.4	42.4	12.1	4.6	35.5	64.5
	2	0.3	1.6	19.6	3.3	3.9	43.4	25.9	2.0	26.0	74.0
	3	0.2	1.2	25.9	6.0	7.8	51.9	7.0	0.0	35.1	64.8
Beef tallow	TG	0.4	2.1	23.9	5.0	8.1	44.5	13.2	2.8	35.3	64.7
	1		2.3	25.5	5.2	8.9	40.4	13.8	3.9	37.5	62.5
	2	0.3	2.3	19.3	3.2	5.5	44.1	22.5	2.8	28.1	71.9
	3	0.9	1.7	26.9	6.6	9.9	49.0	3.3	1.7	40.3	59.8
Breast muscle											
Soybean oil	TG	0.5	1.0	19.1	2.8	5.8	36.3	30.7	3.8	26.8	73.2
	1	0.5	1.1	24.3	3.3	8.4	31.2	23.6	7.6	34.9	65.1
	2	0.7	1.1	14.4	2.2	3.3	32.4	42.1	3.8	20.2	79.8
	3	0.3	0.8	18.6	2.9	5.7	45.3	26.4	0.1	25.4	74.6
Coconut oil	TG	15.6	8.5	19.7	3.5	5.7	31.1	12.9	3.0	50.4	49.6
	1	13.1	9.3	23.3	4.2	7.6	28.7	9.9	3.9	54.1	45.9
	2	12.5	7.4	16.2	2.7	3.4	32.1	23.2	2.5	40.0	60.0
	3	21.2	8.8	19.6	3.6	6.1	32.5	5.6	2.6	57.1	42.9
Lard	TG	0.3	1.4	24.5	4.2	7.4	44.9	15.2	2.1	34.0	66.0
	1	0.3	1.5	27.0	4.9	9.9	40.7	12.1	3.5	39.2	60.9
	2	1.0	1.8	21.0	3.4	4.0	40.6	25.9	2.3	28.6	71.4
	3		0.9	25.5	4.3	8.3	53.4	7.6	0.0	34.4	65.5
Beef tallow	TG	0.8	2.1	23.9	4.0	8.3	43.9	14.3	2.7	35.8	64.2
	1		2.3	25.7	4.7	9.6	37.5	16.9	3.3	38.9	61.1
	2	0.4	2.3	19.2	2.9	5.5	42.7	24.1	2.9	28.2	71.8
	3	2.0	1.7	26.8	4.4	9.8	51.5	1.9	1.9	40.3	59.8

* Stereospecific number in triacylglycerol

** TG=Original triacylglycerol

*** Contained 10:0, 14:1, 15:0, 17:0 Br, 17:0, 17:1, 18:3 and 20:1 fatty acid

Other abbreviations used are the same as those described in Table 1.

soybean oil, coconut oil, lard or beef tallow are presented in Table 2.

Fatty acid composition of breast muscle lipid was similar to that of thigh muscle lipid in each dietary group, although the breast muscle lipid contained a little less oleic acid ($C_{18:1}$) and more arachidonic acid ($C_{20:4}$) than the thigh muscle lipids in each corresponding dietary treatment group.

As shown in Table 2, the muscle lipid from hens fed soybean oil-supplemented diet (soybean oil group) had more linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) than those from the other dietary groups. The muscle lipids from hens fed coconut oil-supplemented diet (coconut oil group) and from soybean oil group had less $C_{18:1}$ than those from lard or beef tallow

feeding groups (lard group or tallow group). The lipids of coconut oil group contained considerably large amount of lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$) compared with those of other dietary groups. Fatty acid compositions of the thigh and breast muscle lipids from lard group were rather similar to that from the tallow group in each muscle. In general, the fatty acid composition in each dietary group reflected the fatty acid patterns of fats supplemented to the feeds.

The results of this study on coconut oil feeding to hens agreed with the changes in fatty acid compositions of chicken muscle lipids observed by MARION *et al.*²⁵⁾, and the results on beef tallow feeding resembled to that reported by SCHULER and ESSARY⁷⁾. The

Table 4 Proportional distributions of fatty acids on each position in a triacylglycerol of chicken muscle lipids (%)

Treatment	Thigh muscle											
	SO			CO			L			B		
	1	2	3	1	2	3	1	2	3	1	2	3
Position												
12:0	69	31		29	25	46	44	33	22		25	75
14:0	53	33	13	37	31	32	33	38	29	37	37	27
16:0	48	22	30	40	26	34	35	28	37	36	27	38
16:1	51	22	27	41	25	34	40	21	38	35	21	44
18:0	47	18	35	46	22	32	42	19	39	37	23	41
18:1	32	31	37	31	35	34	31	32	38	30	33	37
18:2	19	50	31	28	62	9	27	58	16	35	57	8
Sat. acid	48	22	30	35	26	39	37	27	36	35	27	38
Unsat. acid	28	38	34	31	41	28	32	36	32	32	37	31

Treatment	Breast muscle											
	SO			CO			L			B		
	1	2	3	1	2	3	1	2	3	1	2	3
Position												
12:0	32	47	20	28	27	45	23	77			17	83
14:0	37	37	27	36	29	35	36	43	21	37	37	27
16:0	42	25	32	39	27	33	37	29	35	36	27	37
16:1	39	26	35	40	26	34	39	27	34	39	24	37
18:0	48	19	33	44	20	36	45	18	37	39	22	39
18:1	29	30	42	31	34	35	30	30	40	28	32	39
18:2	26	46	29	26	60	14	27	57	17	39	56	4
Sat. acid	43	25	32	36	26	38	38	28	34	36	26	38
Unsat. acid	30	36	34	31	40	29	31	36	33	32	37	31

Abbreviations used are the same as those described in Table 1.

results on soybean oil feeding in this study were similar to the results on feeding of corn oil to hens studied by CHUNG et al.⁸⁾; this was probably due to the similar fatty acid pattern of soybean oil to that of corn oil.

Effects of dietary fats on the fatty acid distribution in TG of muscle lipids of laying hen

The TG of thigh and breast muscle lipids were subjected to stereospecific analyses to investigate the distribution of fatty acids in positions sn-1, sn-2, and sn-3 of TG molecule. The fatty acid composition of original TG and of the three positions of the sn-glycerol moiety are given in Table 3.

In TG of the thigh and breast muscle lipids from hens of all groups, palmitic acid ($C_{16:0}$),

palmitoleic acid ($C_{16:1}$) and stearic acid ($C_{18:0}$) were preferentially esterified in position sn-1 and sn-3. $C_{18:1}$ was generally predominant at position sn-3 excepting in the thigh muscle TG of coconut oil group. Position sn-2 of muscle TG, in both thigh and breast, was preferentially occupied by $C_{18:2}$. Concerning the shorter chain fatty acids of muscle TG from hens fed coconut oil, $C_{12:0}$ was esterified predominantly in position sn-3, whereas $C_{14:0}$ was distributed comparatively evenly into three positions of TG molecule. In all, saturated fatty acids were found more in positions sn-1 and sn-3 than in position sn-2, and unsaturated fatty acids were present in abundance in position sn-2 of chicken muscle TG.

The proportional distributions of fatty acids among three positions in the muscle TG are shown in Table 4.

Proportional distribution patterns of every fatty acids, excepting $C_{12:0}$ and $C_{18:2}$, were comparatively similar in both thigh and breast

Table 5 Triacylglycerol compositions of chicken muscle lipids (mole %)

TG type*	Thigh muscle			
	SO	CO	L	B
sn-SSS	1.8	13.5	3.2	4.2
sn-SSU	5.3	9.0	6.0	6.4
sn-SUS	8.2	19.8	9.2	10.9
sn-USS	2.7	10.9	5.9	7.1
sn-SUU	24.5	13.1	17.1	16.3
sn-USU	8.1	7.2	11.0	10.6
sn-UUS	12.4	15.9	16.8	18.1
sn-UUU	37.0	10.5	31.2	27.1

TG type*	Breast muscle			
	SO	CO	L	B
sn-SSS	1.8	12.4	3.9	4.4
sn-SSU	5.3	9.5	7.4	6.6
sn-SUS	7.1	18.5	9.8	11.3
sn-USS	3.3	10.5	6.1	6.9
sn-SUU	21.0	14.2	18.6	16.7
sn-USU	9.9	8.1	11.6	10.3
sn-UUS	13.2	15.7	15.2	17.7
sn-UUU	39.1	12.1	28.9	26.2

* Calculated triacylglycerol type by means of 1-random, 2-random, 3-random distribution. an- : stereospecifically numbered. S : Saturated, U : unsaturated fatty acid. Other abbreviations used are the same as those described in Table 1.

Fig. 1 GLC separation of triacylglycerols of thigh muscle from laying hens fed fat-supplemented diets. Figures of peaks represent the total number of acyl carbon atoms.

Hitachi Model-163 Gas Chromatograph; Column, 2% Silicon SE-30 on 80-100 mesh Chromosorb WAW; injection and detector's temperature, 330°C; temperature programmed at 7.5°C/min over the range of 200 ~ 300°C.

muscle TG regardless of different dietary treatments. Although, feeding of soybean oil to hen increased the proportion of C_{18:2} distributed in sn-3 position of muscle TG, predominant distribution of C_{18:2} in sn-2 position of TG was common to the muscle TG from all dietary groups. This suggests that the species specificity of positional distribution of main fatty acids in chicken muscle TG is not affected by dietary fats fed to hens.

Effects of dietary treatments of laying hen on the composition and types of muscle TG

The amounts of each TG type in lipid could be calculated from the known fatty acid composition in intact TG assuming a 1-random, 2-random, 3-random arrangement. The results obtained on the thigh and breast muscle TG from each dietary group are given in Table 5.

The proportions of TG types of the thigh muscle lipids were similar to that of the breast muscle lipids in each corresponding dietary group. The most abundant TG type in lipids from soybean oil, lard and tallow groups was triunsaturated TG (sn-UUU), followed by monosaturated diunsaturated TG of sn-SUU or sn-UUS, and the least TG was trisaturated TG

(sn-SSS). In contrast to this, the major component TG types of coconut oil group were sn-SUS, sn-UUS, sn-SUU and sn-SSS, and the lipid of coconut oil group contained the least of sn-UUU among all dietary groups. Muscle lipids from lard group had similar proportions of TG composition to that from tallow group.

Effects of dietary treatments of laying hen on the muscle TG species based on carbon number

The TG was separated into simpler molecular species by high temperature gas-liquid chromatography according to the total number of acyl carbon atoms of TG. The GLC elution patterns for the thigh and breast muscle TG of laying hen from each group are shown in Fig. 1 and 2.

The elution patterns for TG of the thigh muscle lipids from each dietary group were similar to those of breast muscle lipids from corresponding dietary group, respectively. The chromatograms for the muscle TG from soy-

Fig. 2 GLC separation of triacylglycerols of breast muscle from laying hens fed fat-supplemented diets

Figures of peaks represent the total number of acyl carbon atoms. Instrument and operating conditions are the same as the footnote in Fig. 1.

Fig. 3 Argentation thin-layer chromatograms of triacylglycerols of muscle lipids from hens fed fat-supplemented diets

Plate: Silica Gel G impregnated 10% AgNO₃. Developing solvent system: hexane-ethyl ether-benzen-methanol (70/10/20/1, by volume). SO, CO, L and B are soybean oil, coconut oil, lard and beef tallow feeding groups, respectively. S = Saturated, M = monoenoic, D = dienoic and T = trienoic fatty acid moieties.

bean oil, lard and beef tallow groups showed commonly the presence of the TG with acyl carbon number 50, 52 and 54 (C_{50} , C_{52} and C_{54}) as the major TG components, and concomitant minor TG species of C_{44} , C_{46} and C_{48} . On the contrary, the GLC elution patterns of both thigh and breast muscle lipids from coconut oil group, showed the presence of many peaks of medium chain length TG species of $C_{34} \sim C_{48}$ in addition to the $C_{50} \sim C_{54}$.

Distributions of TG types specified by carbon number in the thigh and breast muscle lipids

determined by GLC are given in Table 6. Added to the determined values of TG, the predicted values which were calculated by the positional distribution of fatty acids assuming a 1-random, 2-random, 3-random arrangement are given in Table 6.

The observed and also the predicted values of TG of the thigh muscle lipids from each dietary group were similar to those of breast muscle lipids from corresponding group, respectively. The comparison between the findings by GLC resolution and that by calculation based on

Table 6 Composition of observed and predicted values of triacylglycerols of chicken muscle lipids (wt. %)

TG*** type	Thigh muscle							
	SO		CO		L		B	
	Found*	Calc.**	Found	Calc.	Found	Calc.	Found	Calc.
36			2.7	0.6				
38			3.6	1.2				
40			5.0	2.1				
42			8.0	5.5				
44	0.4	0.1	8.2	9.6	0.4	0.1	0.2	0.2
46	0.7	0.6	10.5	15.3	0.9	0.8	1.0	1.2
48	2.7	1.8	13.2	20.6	4.5	4.5	5.5	5.8
50	14.6	13.5	13.1	14.2	19.1	18.9	20.6	20.0
52	40.4	39.6	19.8	15.0	41.8	40.2	41.2	38.8
54	41.0	40.5	15.6	10.5	33.4	32.6	31.6	29.7
TG type	Breast muscle							
	SO		CO		L		B	
	Found	Calc.	Found	Calc.	Found	Calc.	Found	Calc.
36			1.6	0.4				
38			2.4	0.7				
40			3.6	2.1				
42			5.9	3.7				
44	0.4	0.1	6.2	7.8	0.4	0.3	0.3	0.3
46	0.9	0.5	8.4	14.0	1.1	1.0	1.2	1.5
48	3.5	2.8	11.4	20.0	5.0	4.5	5.6	5.8
50	15.1	12.6	15.1	15.7	19.5	18.7	20.1	18.8
52	39.1	37.9	25.0	18.1	41.7	40.3	40.6	38.2
54	41.0	43.2	20.2	13.2	32.3	32.5	32.3	31.0

* Determined by direct gas-liquid chromatography of triacylglycerol

** Calculated by means of 1-random, 2-random, 3-random distribution

*** Total number of acyl carbon atoms.

Other abbreviations used are the same as those described in Table 1.

random distribution came to a considerably good agreement on the thigh and breast muscle lipids from soybean oil, lard and beef tallow groups. But some discrepancies were observed among the values obtained from different methods on muscle TG from coconut oil group. The experimental values for C_{46} and C_{48} components of muscle lipids, in both thigh and breast, were lower than the predicted values, and the experimental values for the C_{52} and C_{54} components are higher than the predicted values on the muscle TG from coconut oil group.

The findings mentioned above suggest that a 1-random, 2-random, 3-random distribution of fatty acids in TG molecule in relation to those chain lengths occurred in the course of TG synthesis in muscle tissues on the feeding of soybean oil, lard and beef tallow. But, on the feeding of coconut oil, there is preferential synthesis of the C_{52} and C_{54} components, *i.e.* 16, 18, 18-TG and 18, 18, 18-TG, in relation to that for the TG of C_{48} component.

Effects of dietary treatments of laying hen on the muscle TG species based on degree of unsaturation

The TG was separated into simpler molecular

species by $AgNO_3$ -TLC on the basis of total number and distribution of double bonds per molecule. The results are shown in Fig. 3.

Usually nine or more bands of TG species were detected on the plates. Some of those TG were separated in individual bands, but for the instance, the mixture of TG species of S_2D and M_3 , and sometimes the mixture of M_3 and SMD were obtained as a single band on the chromatogram as pointed out by CHRISTIE and MOORE previously²⁶⁾. In such case, the composition of those bands that contain mixture of TG species were calculated on the basis of their fatty acid compositions²⁷⁾. The results of quantitative analysis on TG components of thigh and breast muscle lipids from each dietary group are shown in Table 7.

The muscle lipid from soybean oil group contained less amounts of TG comprising monoenoic acids (S_2M and SM_2), and more of TG comprising dienoic or trienoic acids (M_2D , SD_2 , SMT and D_3) than those from the other groups, while lipid from coconut oil group had most amounts of S_3 and S_2M species among the dietary groups. Although the thigh muscle lipids from both lard and beef tallow groups

Table 7 Triacylglycerol types of chicken muscle lipids from hens fed fat-supplemented diets (mole %)

TG* type	Thigh muscle				Breast muscle			
	SO	CO	L	B	SO	CO	L	B
S_3	2.5	12.7	3.4	3.9	2.6	13.9	4.1	4.0
S_2M	10.5	28.1	15.3	18.8	10.3	23.2	17.0	16.6
SM_2	14.7	17.4	23.0	23.7	13.6	15.8	22.4	26.0
S_2D	8.7	7.9	6.7	5.7	6.0	9.0	7.0	6.9
M_3	8.6	6.9	16.5	15.9	6.4	6.8	12.2	10.9
SMD	19.5	17.7	22.6	19.3	19.5	17.6	18.7	16.3
M_2D	11.5	5.4	8.3	8.1	11.7	7.5	12.1	12.5
SD_2	8.2	2.4	2.7	1.8	8.7	3.5	3.0	2.5
S_2T	2.2	1.3	1.2	1.5	1.2	1.7	1.7	1.6
MD_2	6.7		0.1	1.0	8.6	0.4	1.2	2.1
SMT	3.2	0.2	0.2	0.3	5.7	0.7	0.6	0.6
D_3	3.4				5.2			
SDT	0.3				0.5			

*S=Saturated, M=monoenoic, D=dienoic and T=trienoic fatty acid.
Other abbreviations used are the same as those described in Table 1.

Table 8 Comparison of observed and predicted values of triacylglycerols of chicken muscle lipids (mole %)

TG type*		Thigh muscle			
		SO	CO	L	B
S ₃	Found	2.5	12.7	3.4	3.9
	Calc.	1.8	13.5	3.2	4.2
S ₂ U	Found	21.4	37.3	23.2	26.0
	Calc.	16.2	39.7	21.1	24.4
SU ₂	Found	45.9	37.7	48.5	45.1
	Calc.	35.0	36.2	44.9	45.0
U ₃	Found	29.2	12.3	24.5	24.9
	Calc.	37.0	10.5	31.2	27.1

TG type*		Breast musele			
		SO	CO	L	B
S ₃	Found	2.6	13.9	4.1	4.0
	Calc.	1.8	12.4	3.9	4.4
S ₂ U	Found	17.5	33.9	25.7	25.1
	Calc.	15.7	38.5	23.3	24.8
SU ₂	Found	48.0	37.6	44.7	45.4
	Calc.	44.1	38.0	45.4	44.7
U ₃	Found	31.9	14.7	25.5	25.5
	Calc.	39.1	12.1	28.9	26.2

*S=Saturated and U=unsaturated fatty acid
Other abbreviations used are the same as those described in Table 1.

had more M₃ and SMD species and less M₂D than the breast muscle lipids from each corresponding dietary group, the muscle TG from both groups showed comparatively similar values in each TG species in each muscle tissue.

Table 8 compares the values of TG species found by AgNO₃-TLC with those calculated by a 1-random, 2-random, 3-random distribution of fatty acids in TG of muscle lipids from each dietary group.

Considerable good agreements were observed between the actual and predicted values in muscle lipids from coconut oil, lard and tallow groups, except U₃ species of the thigh muscle from lard group. In the muscle lipids from soybean oil group, some differences between determined and calculated values were observed in TG of SU₂ and U₃ species. This findings that the experimental values were higher for

TG of SU₂ and lower for U₃ than the random values suggest the preferential synthesis of the diunsaturated TG in relation to that of the triunsaturated TG in the laying hens on feeding of soybean oil-supplemented diet.

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産卵鶏筋肉脂質のトリアシルグリセロールの
組成ならびに構造に及ぼす
飼料油脂の影響

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産卵鶏筋肉脂質のトリアシルグリセロール (TG) の
組成と構造に及ぼす飼料油脂 (大豆油, ココヤシ油, ラ

ードおよび牛脂) の影響を調べた。各飼料区の筋肉総脂質の脂肪酸組成は、飼料に加えた油脂の組成を反映していた。腿肉および胸筋の TG を立体特異的分析に付した。一般に C_{16:0}, C_{16:1}, C_{18:0} は TG の sn-1 および sn-3 位置に優先的にエステル化していた。C_{18:1} は sn-3 位置に多く、C_{18:2} は sn-2 位置に多く結合していた。高温ガスクロマトグラフィーにより、炭素数に基づき TG を分別した。大豆油区、ラード区および牛脂区の脂質は、共に C₅₀, C₅₂ ならびに C₅₄ を主要な TG 成分とし、ココヤシ油区の脂質は、C₅₀ ~ C₅₄ に加えて中鎖炭素数の C₃₄ ~ C₄₈ の TG 成分を多く有していた。硝酸銀付加薄層クロマトグラフィーによって、不飽和度に基いて TG 分子種を分画した。大豆油区の脂質は、他の飼料区の脂質よりも S₂M や SM₂ が少なく、M₂D, SD₂, MD₂, SMT および D₃ 成分が多かった。これに対してココヤシ油区の脂質は、飼料区のなかでは S₃ と S₂M 成分が最も多かった。TG 分子種の実測値と、TG 内で脂肪酸の 1-ランダム、2-ランダム、3-ランダム配置を仮定して求めた計算値との比較を行った。ラード区と牛脂区の筋肉脂質にあつては、比較的良く一致していたが、大豆油区やココヤシ油区の脂質では、相違の認められるところがあった。このことは、摂取した飼料脂肪酸に影響され、産卵鶏では TG の生合成にあたって、ある種のタイプの TG 分子種の優先的な合成も行われることを示唆している。