Alterations in Lipid Metabolism between the Growing and the Laying Periods of White Leghorn Layers

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ABSTRACT : This study compared the lipid metabolism of white Leghorn layers between the growing and the laying periods. The study was conducted on thirty layers in the growing period (14 weeks old) and in the initial laying period (36 weeks old; 14 weeks from the onset of laying). Results indicated that all plasma lipid traits were significantly different ($p<0.05$) between the two periods. The estrogen concentration in the laying period was about 3 fold that in the growing period. Triacylglycerol (TG) concentration in the laying period was about 12 fold that in the growing period. The phospholipids, cholesterol, glycerol and nonesterified fatty acid (NEFA) in the laying period were also higher than those in the growing period ($p<0.05$). Lipogenic enzyme activities in the laying period were higher than in the growing period ($p<0.05$). High-density lipoprotein (HDL) was the largest lipoprotein portion in growing layers. In laying hens, the major lipoprotein portion was very low-density lipoprotein (VLDL). It was also shown that protein is the primary component of HDL and TG is the largest portion of VLDL in both periods. In laying hens, VLDL contained more TG than that in growing layers ($p<0.05$). The VLDL particle size in laying hens was larger than that in growing layers ($p<0.05$). This study indicates that the lipid metabolism traits of a layer in the laying period were different from those in the growing period. The lipogenesis related traits in the laying period were markedly greater than in the growing period. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 10 : 1460-1464)*

Key Words : Leghorn Layers, Lipid Metabolism, Growing and Laying Periods

INTRODUCTION

The lipid metabolism of birds might alter between the laying period and the growing period, because laying avian follicles secrete estrogen. The administration of estrogen is frequently associated with hypertriglyceridemia and a relatively increased VLDL level (Park and Cho, 1988). The avian ovary lacks the capacity to synthesize lipids and, therefore, all of the lipid components in the yolk are derived primarily from a plasma precursor such as VLDL synthesized in the liver. The liver is the major site for lipogenesis in avians. Hepatic lipogenesis is dramatically stimulated by estrogen (Chapman, 1980; Hasegawa et al.,1980a, 1980b, 1982; Tanaka et al., 1986). Thus, the lipid metabolism of layer in the laying period might be different from that in the growing period. Chapman (1980) and Hermier et al. (1985) indicated that HDL is the largest portion of the lipoprotein in the poultry growing period, while in the laying period the major lipoprotein is VLDL. In this study, we compared the plasma traits and the lipogenic enzyme activities, lipoprotein profile, chemical components and particle size in white Leghorn layers during the growing and the laying periods.

MATERIALS AND METHODS

Animal and treatment

Thirty layers were used to compare lipid metabolism between the growing and the laying periods. Layers were caged (45×30×35 cm) individually. Ambient temperatures in the day-time were 30.67±2.32°C and 28.32±2.67°C in the growing and the laying periods, respectively. Water was available at all times. The diet composition is shown in table 1. During the growing period (10-14 weeks of age) and the initial laying period (32-36 weeks of age; 10-14 weeks from the onset of laying). Layers were restricted to feeding at 8:30 and 16:30. The feeding levels were 80 and 110 g during the growing and laying periods, respectively. The final body weights and growth rates during the growing and the laying periods were 1.04 kg, 240 g/30 days and 1.48 kg, 120 g/30 days, respectively. In the laying period, the laying performance was 84.26±2.37% (hen day basis). Layers were fasted overnight, and then blood samples were taken from the wing vein with 15% EDTA and plasma was isolated for analysis. Finally, layers were sacrificed and liver samples taken to determine lipogenic enzyme activities.

Sample preparation

Blood samples were centrifuged at 2000×*g* for 15 min to obtain plasma. Fresh liver samples (5 g) were homogenized with 15 ml of ice-cooled 5 M Tris-HCl buffer (pH 7.2) containing 0.25 M sucrose and centrifuged at 800 ×*g* for 10 min to remove debris. The supernatant fraction was centrifuged at 15.000×*g* for 10 min. After discarding the pellets, the supernatant was centrifuged at 105,000×*g* for 60 min (SW 41 rotor, Beckman, Model L-8, USA). The supernatant fraction (cytosol) was taken for further assay.

The liver cytosol isolation procedures were performed at 0-4°C and samples were stored below -70°C until the enzyme activities were measured.

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Table 1. Composition of basal diets

Ingredients	Growing period	Laying period
	%	
Yellow corn meal	68.37	60.02
Soybean meal, 44%	16.27	23.52
Fish meal, 65%	2.00	3.00
Wheat bran	10.00	-
Soybean oil	-	3.01
Dicalcium phosphate	1.84	1.25
Limestone, pulverized	0.60	4.26
Salt	0.30	0.30
Vitamin premix [(1)]	0.03	0.03
Mineral premix [(2)]	0.10	0.10
Oyster shells	-	4.00
DL-Methionine	-	0.01
Choline chloride, 50%	0.40	0.50
Total	100.00	100.00
Calculated value :		
Crude protein, %	16.00	17.50
ME, kcal kg^{-1}	2,840	2,840
Calcium, %	0.90	3.40
Available phosphorus, %	0.50	0.40
Lysine, %	0.79	0.96
Methionine, %	0.27	0.30
Methionine+Cystine, %	0.50	0.55
Choline, mg kg^{-1}	1,016	1,295

[(1)] Vitamin premix supplied the following per kilogram of diet : Vitamin A, 25,000 IU ; Vitamin D_3, 3,125 ICU; Vitamin E, 37.5 IU; Vitamin K_3, 6.25 mg; Vitamin B_1, 3.75 mg; Vitamin B_2, 12.5 mg; Vitamin B_6, 10.0 mg; Pantothenate, 18.8 mg; Niacin, 50 mg; Biotin, 0.06 mg; Folic acid, 1.25 mg; Vitamin B_{12}, 0.05 mg.

[(2)] Mineral premix supplied the following per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu) 6 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.29% Fe) 50 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn) 40 mg; Zn (ZnO, 80.35% Zn) 60 mg; Se (Na_2SeO_3, 45.56% Se) 0.075 mg.

Lipogenic enzyme activities determination

To determine lipogenic enzyme activity, the liver cytosol samples were added to the medium and measured using a spectrometer (Hitachi, U2000, Japan) at 340 nm in 25°C for 5 minutes. Acetyl-CoA carboxylase (EC 6.4.1.2, ACC) was determined according to the method of Numa (1969). ATP-citrate cleavage enzyme (EC 4.1.3.8, CCE) was analyzed based on the methods described by Takada et al. (1963). NADP-malic dehydrogenase (EC 1.1.1.40, MDH) was determined according to the method of Ochoa (1955). Fatty acid synthetase (EC 2.3.1.85, FAS) was measured following the method of Kumar et al. (1970). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G-6-PDH) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6-GPDH) were measured following the method of Lohr and Waller (1974).

Lipoprotein isolation and determination

Plasma lipoprotein isolation was arranged according to the Redgrave et al. (1975) procedure, that is different gradient density solutions were added and subjected to ultracentrifugation at 40,000 rpm (105,000×*g*) for 24 h at 15°C with a SW-41 swinging bucket rotor (Beckman, L8, USA). The various lipoprotein fractions were then collected. Each lipoprotein fraction was verified by electrophoresis (Helena, Co. USA). The electrophoresis method was also employed for plasma lipoprotein distribution analysis. Each concentration was determined using a densitometer (Helena, Model: BJf00105, USA) at 525 nm.

Plasma traits and lipoprotein components determination

Plasma nonesterified fatty acid (NEFA) was measured according to the procedure developed by Chromy et al (1977). Total cholesterol and triacylglycerol (TG) were determined with an enzyme kit using an automatic serum biochemical analyzer (Roche, Co. Switzerland). Glycerol and phospholipids were determined by Imai's (1967) procedures. Plasma estrogen was measure by radioimmunoassay method (Nakamura et al., 1974). The protein content was analyzed by Lowry method (Lowry, 1951). Phospholipid was measured by the Imai (1967) method.

The lipoprotein particle diameter was determined by placing one drop of lipoprotein solution onto a formvar carbon-coated grid membrane, then treating it with a negative stain using 2% phosphotungstic acid. After drying, the lipoprotein particles were viewed with a transmission electron microscope (TEM) (Hitachi, H-7100, Japan).

Statistical analysis

Data were analyzed to identify the variance between groups with a general linear model using SAS software. Significant differences were then compared using the Tukey's test (SAS, 1998).

RESULTS

The differences in plasma traits in the growing and the laying periods are shown in table 2. All plasma traits were significantly different ($p<0.05$) between the growing and the laying periods. The estrogen concentration in the laying period was about 3 fold that in the growing period. The triacylglycerol concentration in the laying period was about 12 fold that in the growing period. The phospholipids, cholesterol, glycerol and NEFA levels in the laying period were also higher than in the growing period. ($p<0.05$).

The differences in lipogenesis related enzyme activities in the growing and in the laying periods are displayed in table 3. This table reveals that the lipogenic enzyme activities in the laying period were higher than in the

Table 2. Plasma traits in the growing and the laying periods of White Leghorn layers

Items	Growing period	Laying period
Phospholipids, mg/dl	76.1±2.7	136.26±8.1*
Triacylglycerol, mg/dl	121.9±6.8	1,583.8±105.5*
Cholesterol, mg/dl	97.2±2.5	117.2±3.8*
Nonesterified fatty acid, mg/dl	17.0±0.9	27.8±1.7*
Glycerol, mg/dl	52.4±4.2	70.7±1.8*
Estrogen, pg/dl	29.2±1.3	83.6±2.1*

Means±SE (n=30)
*: Mean in the same row different significantly ($p<0.05$)

growing period ($p<0.05$).

Table 4 presents the plasma lipoprotein distribution of layers in the growing and the laying periods. This table reveals that HDL is the largest component of lipoproteins in the growing period and is significantly greater than in the laying period ($p<0.05$). The VLDL fraction was markedly higher in laying hens and significantly larger than in growing layers ($p<0.05$). The LDL fraction quantity in the laying period was small and, therefore, a sufficient amount for composition analysis could not be obtained. The VLDL particle in the laying period was larger than that in the growing period ($p<0.05$). The HDL particle size in the laying period could not be determined because of insufficient sample.

As shown in table 5, protein is the largest component of HDL. The protein content in HDL in the growing period was significantly higher than that in the laying period. In contrast, the cholesterol content of HDL in laying hens was the largest component, and higher than in the growing period ($p<0.05$).

Table 3. Enzyme activities related to lipogenesis in the growing and the laying periods of White Leghorn layers

Items	Growing period	Laying period
	Unit	
ACC	0.8±0.1	2.0±0.2*
FAS	10.5±0.9	36.2±4.3*
CCE	76.4±6.2	116.9±6.4*
MDH	316.6±14.2	503.1±22.6*
G-6-PDH	6.9±0.4	44.4±5.6*
6-PGDH	11.9±0.4	15.8±1.3*

Means±SE (n=30)
*: Mean in the same row different significantly ($p<0.05$)
1 Unit=1 nM/mg protein
ACC: Acetyl-CoA carboxylase
FAS: Fatty acid synthetase
CCE: ATP-Citrate cleavage enzyme
MDH: NADP-Malic dehydrogenase
G-6-PDH: Glucose-6-phosphate dehydrogenase
6-PGDH: 6-Phosphogluconate dehydrogenase

Table 6 compares the plasma VLDL composition of layers between the growing and the laying periods. As shown in table 6, TG is the major portion of VLDL in both the growing and the laying periods. However, the TG component of VLDL in laying hens was significantly greater than that in growing layers ($p<0.05$). The cholesterol concentration of VLDL in growing layers was higher than that in laying hens ($p<0.05$).

DISCUSSION

When birds are laying, the ovarian follicles secrete estrogen to stimulate liver lipogenesis. This event obviously enhances plasma TG and phospholipid concentrations (Hasegawa et al., 1980b, 1982; Leclercq, 1984; Tanaka et al., 1986). This study demonstrated that the activities of liver lipogenic enzymes in layers are higher during the laying period than the growing period, which is in

Table 4. Lipoprotein profile and particle size in the growing and the laying periods of White Leghorn layers

Items	Growing period	Laying period
HDL, %	50.7±1.5	4.1±0.5*
LDL, %	32.6±1.2	---
VLDL, %	16.7±1.3	95.9±0.5*
HDL particle, nm	10.7±0.8	---
VLDL particle, nm	89.4±5.6	141.5±5.0*

Means±SE (n=30)
*: Mean in the same row different significantly ($p<0.05$).
---: Mean undetermined.

Table 5. High-density lipoprotein components in the growing and the laying periods of White Leghorn layers

Items	Growing period	Laying period
	%	
Protein	45.0±1.3	4.1±0.5*
Cholesterol	17.1±1.4	31.7±2.9*
Triacylglycerol	10.7±1.4	14.2±2.4
Phospholipids	27.9±1.4	26.5±2.1

Means±SE (n=30)
*: Mean in the same row different significantly ($p<0.05$).

Table 6. Very low-density lipoprotein components in the growing and the laying periods of White Leghorn layers

Items	Growing period	Laying period
	%	
Protein	23.6±3.3	23.8±1.7
Cholesterol	24.6±3.9	4.3±0.7*
Triacylglycerol	41.0±4.8	67.2±2.6*
Phospholipids	10.8±4.7	5.6±0.8

Means±SE (n=30).
*: Mean in the same row different significantly ($p<0.05$).

agreement with our previous study with ducks (Lien et al., 1999). A bird during the laying period, or after an injection of estrogen, would have depressed adipose tissue LPL activity and an increased plasma VLDL level, and slow plasma TG clearance (Hasegawa, 1980a; Tanaka et al., 1986). That results in enhanced plasma TG.

Chapman (1980) and Hermier et al. (1985, 1989) indicated that the plasma lipoprotein distribution in chickens was somewhat different from that in mammals. In humans, LDL is the largest portion of plasma lipoprotein. In chickens, the largest portion of plasma lipoprotein in the growing period is HDL. This study obtained a similar result. In the laying period, VLDL concentration was relatively high (Hermier et al., 1989), while the HDL level decreased. Park and Cho (1988, 1990) treated roosters with estrogen, and found that their plasma lipid traits changed substantially. Plasma TG levels rose dramatically, and the VLDL fraction apparently increased. In addition, the VLDL particle sizes also enlarged. Our results are very similar to those of Park and Cho (1988, 1990). Our previous study demonstrated that the plasma TG and VLDL levels in ducks during the laying period were many fold greater than those in the growing period (Lien et al., 1999). This change was due to follicles secreting estrogen which stimulated liver lipogenesis (Yu et al., 1976; Bacon et al., 1978; Bacon, 1981; Griffin et al., 1982).

The chemical composition of various lipoproteins revealed some differences between laying and growing layers. Laying birds contain more TG than growing birds, particularly in their plasma VLDL (Griffin et al., 1982). Granfone et al. (1992) indicated that human oral estrogen could increase VLDL. Meanwhile, HDL-cholesterol displayed an increase. Those findings are consistent with the results obtained in this study.

Bacon et al. (1978) reported that the VLDL turnover rate of laying hens showed a decrease. Compared to growing chickens, their TG degradation rate reduced since their lipoprotein lipase activator apolipoprotein, the apo C-II, was lower than in growing chickens. In our previous study, the apo C-II concentration in growing ducks was higher than that in laying ducks (Lien et al., 1998). That leads to the lipoprotein lipase being less active so that most of the VLDL-TG could be slowly transferred into the yolk (Leclercq, 1984). This study reveals that TG content in the VLDL of laying hens is greater than that in growing layers, which may be the result of the VLDL-TG degradation rate by lipoprotein lipase in laying hens being slower than that in growing layers.

The difference in particle size in the lipoprotein was mainly in the core of the particle, since TG is the major component of the core. The VLDL-TG in the laying period was greater than that in the growing period, thus, the VLDL particle size in laying hens was larger than that in growing layers. In growing layers, the VLDL contains more TG than HDL, therefore, the VLDL particle size was larger than that of HDL.

In conclusion, this study indicated that the lipid metabolism of layers in the laying period was different from that in the growing period. The lipogenesis related traits in the laying period were markedly greater than in the growing period.

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