The Lipogenic Capacity of Hepatocytes and Lipolytic Rate of Adipocytes in Tsaiya Ducks during Growing and Laying Periods

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ABSTRACT : With an attempt to elucidating the lipid metabolism of Tsaiya ducks, thirty ducks at growing (8 weeks of age) and laying periods (10 weeks after the onset of laying) were examined, respectively. The ducks were randomly allocated into *ad libitum* feeding and 3-day fasting groups, to investigate their *in vitro* hepatocytes lipogenesis capacity and adipocytes lipolysis rate. Results indicate that (1) the capacity of hepatocytes incorporation of glucose and acetate into total lipid and metabolite of $^{14}CO_2$ production during the laying period was greater than during the growing period. Approximately 50% of the glucose or acetate converted into triacylglycerol (TG) by the hepatocytes were recovered as fatty acid during the growing period, while it was 65-70% during the laying period. (2) Acetate used for lipogenesis ability was superior to glucose in both periods. (3) The adipocytes lipolysis rate was increased significantly ($p<0.05$) by fasting. In contrast, the capacity of incorporated glucose or acetate into total lipid, triacylglycerol, fatty acid and glycerol by hepatocytes was reduced significantly ($p<0.05$) by fasting. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 8 : 1258-1262*)

Key Words : Tsaiya Ducks, Lipogenesis, Lipolysis, Hepatocytes, Adipocytes, Growing Period, Laying Period

INTRODUCTION

Despite the prominence of duck production in Taiwan poultry industry, information pertaining to a ducks lipid metabolism is scarce, despite the fact that lipid metabolism in chickens has been extensively studied. Owing to the lack of data, chickens lipid metabolism data are frequently used for reference to ducks. Since chicken is a land fowl and duck is a water fowl, their behaviors markedly different, thus, differences in lipid metabolism between these two kinds of fowl may exist. In a related work, Lee et al. (1985, 1987) have indicated that certain kinds of ducks (e.g. mule duck) could be used for Foie Gras production, while chickens could not be used for this purpose. Therefore, lipid metabolism of ducks seems different from chickens and need to be further investigated and understood.

The lipid metabolism of birds during laying period may differ from that during growing period. That laying birds follicles can secrete estrogen to stimulate lipogenesis, thus, plasma TG markedly elevates in the laying period (Hasegawa et al., 1980a, 1980b, 1982; Tanaka et al., 1986). In addition, plasma lipoprotein profile may also change and indicated that high-density lipoprotein (HDL) is the largest population of lipoprotein in growing birds, and very low-density lipoprotein (VLDL) is the major one in laying birds (Chapman, 1980; Hermier et al., 1985). In this study, the lipid metabolism of Tsaiya ducks with *in vitro* method both during growing and laying periods under *ad libitum* and fasting conditions were investigated.

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MATERIALS AND METHODS

Animal treatment

trial was conducted with 30 Tsaiya ducks (*Anas platyrhynchos var. domestica*) at growing period (8 weeks of age) and laying period (10 weeks after the onset of laying), respectively. Ducks were randomly divided into *ad libitum* feeding and fasting groups. The fasting group was continuously fasted for 3 days before the end of each trial. The diets composition are listed in table 1. Ducks were individually caged, and available feed and water were supplied. The trials lasted for 4 weeks in both periods. At the end of trials, all ducks were sacrificed for isolation of hepatocytes and adipocytes.

Isolation of hepatocytes and adipocytes

1) Isolation of hepatocytes

Ducks were anesthetized with sodium barbital (130 mg/100 ml) (5 ml/kg BW) and then, disinfected with iodine and 70% alcohol. The abdomen then opened by a pair of scissors. The liver was then infused with the 40℃ HBSS (Hanks balanced salt solution) buffer (pH 7.4) (added 2 ml 50 mM EGTA/200 ml buffer) from the portal vein by a pump (40 ml/min). The liver surface was massaged to facilitate flushing out of the blood from the organ. Meanwhile, the inferior vena cava was incised. As the liver turned to a yellow-brown color, the liver was perfused with a HBSS buffer solution (10-20 ml/min) containing 70

mg collagenase (type IV, 125 unit/mg)/200 ml buffer (200 ml HBSS buffer added 2 ml 50 mM $CaCl_2$ and 4 g BSA). The liver was also gently massaged until the liver surface was soft and then, the liver was removed and scraped with No. 22 needle. The suspension solution was filtered through 250 and 100 mesh nylon screens. The hepatocytes was then washed three times with a HBSS buffer solution, centrifuged at 50 g for 3 min and a DMEM (Dulbecco's modified Eagle's medium) (containing 10% fetal calf serum) was added to the washed hepatocytes. Finally, the hepatocytes were stained with trypan blue (1:1) for 3-5 min, in which the cells were counted as the red blood cells examination method.

Table 1. Composition of experiment diets (%)

Ingredients	Growing period	Laying period
Yellow corn meal	64.44	58.72
Soybean meal, 44%	16.43	27.38
Fish meal, 65%	2.00	3.00
Wheat bran	12.00	-
Dicalcium phosphate	2.37	1.48
Limestone, pulverized	0.73	3.00
Salt	0.30	0.30
Vitamin premix[1]	0.03	0.03
Mineral premix[2]	0.10	0.10
Soybean oil	-	1.23
Oyster shells	-	4.00
DL-Methionine	0.10	0.17
Choline chloride, 50%	1.50	0.59
Total	100	100
Calculated value:		
Crude protein, %	16.00	19.00
ME, kcal kg^{-1}	2,730	2,730
Calcium, %	1.10	3.00
Avail. P, %	0.60	0.45
Lysine, %	0.90	1.06
Met.+Cystine, %	0.60	0.75
Choline, mg kg^{-1}	1,754	1,430

[1] Vitamin premix supplied the following per kilogram of diet: Vitamin A, 25000 IU; Vitamin D_3, 3125 IU; 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.

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 ($NaSeO_3$, 45.56% Se) 0.075 mg.

2) Isolation of adipocytes

The fresh abdominal fat tissue of ducks was removed and placed in polypropylene Erlenmeyer flasks. After addition with 1.5 ml of KRB (Krebs-Ringer-bicarbonate)-HEPES buffer containing 3mg collagenase (type I, 324 unit/mg) per g of tissue, the adipose tissue was cut into finely minced pieces by a pair of scissors. The pieces were then incubated with a shaker (90 stroke/min) at 37℃ for 1 h under an atmosphere containing 95% O_2 and 5% CO_2 for digestion. After incubation, filtering was performed with 500 and 250 mesh nylon screens. The adipocytes were carefully washed three times using KRB buffer. Finally, the cell numbers were counted by examination of the hematocrit.

Lipogenesis capacity and lipolysis rate determined *in vitro*

1) Determination of lipogenesis and metabolite of $^{14}CO_2$ production

Approximately 6×10^6 hepatocytes were placed into a tube containing 5ml KRB medium and 0.5 μ *Ci*[U,^{14}C]glucose and 1 μ *Ci*[1,^{14}C]acetate were added, respectively. The hepatocytes were incubated at 37℃ for 2 h with a shaker (90 stroke/min) and air containing 95% O_2 and 5% CO_2 was supplied. After incubation, 0.25 ml 1N H_2SO_4 was added to curtail the reaction and liberate $^{14}CO_2$. A filter paper strip (No 1, 2×10 cm) was placed in the plastic center well and 2 ml 25% KOH was injected onto paper to absorb $^{14}CO_2$. The tube was fitted with a rubber cap and the solution was continuously shaken for 60 min to collect the $^{14}CO_2$ production. Total lipid was extracted from the medium and cells using the method of Folch et al. (1957). Half of the amount of total lipid was taken and mix with a scintillation solution and, finally, the radioactivity was measured using a liquid scintillation counter (Backman, LS3801, USA) (Mersmann and Hu, 1987).

3) Determination of lipolysis rate

Approximately 1×10^6 adipocytes were added into a test tube and combined with 5ml KRB medium (1/2 Ca^{2+} concentration) containing 5.56 mM glucose, 0.56 mM ascorbate, 0.5 mM adenine deaminase and 4% fatty acid-free BSA (fraction V). The stimulated lipolysis was performed in the presence of 0.1 mM isoproterenol birtartrate and 10 mM theophylline, incubated at 37℃ for 2 h with a shaker and air containing 95% O_2, 5% CO_2 was supplied. During the terminal incubation, the tube was immersed into cold water to curtail metabolism. The medium was filtered through two layer of cheesecloth. Then, the non-esterified fatty acid (NEFA) was measured according

to the method of Chromy et al. (1977) to express the rate of lipolysis (Mersmann and Hu, 1987).

Determination of triacylglycerol, fatty acid and glycerol synthesis capacity

1) Determination of triacylglycerol synthesis capacity

Approximately one-half of the total lipid was dissolved in hexane : ethyl acetate (9:1, v/v). The lipid components were separated using TLC plate. The solvent was petroleum ether : ethyl acetate : acetic acid (85:15:2, v/v/v); the color reagent was 50% ethanolic H_2SO_4. Triacylglycerol (TG) standard was run concurrently. The TG band was scraped and ethyl acetate was added to extract TG. After centrifugation, TG fraction was collected and dried by flushing with nitrogen. Then, the radioactivity was counted to measure the TG synthesis capacity.

2) Determination of fatty acid and glycerol synthesis

Half amount of the extracted TG and 10 ml 10% ethanolic KOH were combined in a test tube and incubated at 65±5℃ for 1h for saponification. Then, 10 ml of distilled water was added and pH value was adjusted with 6 N HCl to below 3. The released fatty acid was extracted by 5 ml haxane and dried with nitrogen flushing. Then, the radioactivity was counted (Allee et al., 1971). Glycerol synthesis concentration was calculated from TG deduct of the fatty acid.

Statistical analysis

SAS software was used for all data to analyze the variance between groups. The significance was then determined with Tukey's test (SAS, 1990).

RESULTS

Growing period (8~12 weeks age)

Table 2 presents the lipogenesis capacity of hepatocytes and lipolysis rate of adipocytes of Tsaiya ducks at growing period. Incorporation of [1,^{14}C]acetate into total lipid by hepatocytes was greater ($p<0.05$) than that of [U,^{14}C]glucose. The capacity of hepatocytes incorporation of [U,^{14}C]glucose or [1,^{14}C] acetate into total lipid was reduced significantly ($p<0.05$) by fasting. Metabolite of $^{14}CO_2$ production ability was reduced as well ($p<0.05$). Based on the recovered fatty acids, approximately 50% of the glucose or acetate converted into triacylglycerol by the hepatocytes. The capacity of synthesis TG, fatty acid and glycerol by hepatocytes were markedly ($p<0.05$) decreased by fasting. Moreover, the lipolysis rate of adipocytes was significantly increased ($p<0.05$) by fasting.

Laying period (10-14 weeks after the onset of laying)

The lipogenesis capacity of hepatocytes and lipolysis rate of adipocytes of Tsaiya ducks during the laying period is shown in table 3. Total lipid incorporation rate in laying period was greater than in growing period. The rate in use of [1,^{14}C]acetate converted into total lipid was superior to that in use of [U,^{14}C]glucose. Moreover, the rate of incorporation of glucose or acetate into total lipid was decreased markedly ($p<0.05$) by fasting. Metabolite of $^{14}CO_2$ production from acetate declined as well ($p<0.05$). TG synthesized from acetate was higher than that from glucose. Approximately 60-70% of the glucose or acetate converted into TG by ducks hepatocytes was recovered as fatty acid. However, the amount for glycerol was low. Due to marked ($p<0.05$) decreases of the TG, fatty acid and glycerol incorporation rate, the lipolysis rate of adipocytes was significantly ($p<0.05$) enhanced by fasting.

Table 2. Lipogenesis capacity of hepatocytes and lipolysis rate of adipocytes of Tsaiya ducks at growing period (12 weeks of age)

Items	Fed	Fast
Lipogenesis (dpm/ml/min) :		
Metabolite of $^{14}CO_2$ production :		
Glucose	9,787.81±781.60	7,141.13±468.30*
Acetate	27,926.75±1,747.68	15,527.25±1,351.59*
Total lipid synthesis :		
Glucose	13,559.25±822.59	10,297.00±651.77*
Acetate	96,142.63±10,841.32	35,466.00±4,072.70*
Triacylglycerol :		
Glucose	986.49±60.81	306.56±21.91*
Acetate	743.13±41.38	255.94±17.95*
Fatty acid:		
Glucose	428.38±18.81	144.31±9.27*
Acetate	300.25±17.13	157.75±5.57*
Glycerol :		
Glucose	552.31±43.02	166.06±8.27
Acetate	442.94±22.44	98.19±9.20*
Lipolysis (μM/h) :		
NEFA	1,116.50±111.81	1,880.35±177.12*

Means SE (n=15)

* : Means in the same row are significantly different ($p<0.05$).

DISCUSSION

Lipid metabolism of birds in the laying period differs from that in the growing period, since the follicles of laying birds secrete estrogen that stimulates lipogenesis, it results in a remarkable increase in plasma TG and very low-density lipoprotein (VLDL) level (Husbands and Brown, 1965; Balnave, 1968; Hasegawa et al., 1980a, 1980b, 1982; Leclercq, 1984; Tanaka et al., 1986). Our recent in vivo studies also indicated that the contents of plasma TG and VLDL of laying ducks were higher than those in growing ducks. The activities of liver lipogenesis related enzymes were also higher (unpublished data). Results presented herein also demonstrate that hepatocytes lipogenic rate from glucose or acetate in laying ducks were greater than the growing ducks. Our result was in agreement with the report of Evans (1972) that 75% of the glucose converted into total lipid by ducks liver was recovered as fatty acid.

Table 3. Lipogenesis capacity of hepatocytes and lipolysis rate of adipocytes of Tsaiya ducks at laying period (14 weeks after the onset of laying)

Items	Fed	Fast
Lipogenesis (dpm/ml/min) :		
Metabolite of $^{14}CO_2$ production :		
Glucose	18,788.30±1418.95	16,580.93±918.09
Acetate	75,512.67±3,841.60	52,237.33±3,307.83*
Total lipid synthesis :		
Glucose	21,056.63±2,035.69	11,912.80±1,965.94*
Acetate	116,912.87±9,189.05	64,830.07±7,156.84*
Triacylglycerol :		
Glucose	776.60±46.92	638.80±29.65*
Acetate	2,303.14±310.37	355.12±35.20*
Fatty acid:		
Glucose	561.20±24.62	464.80±28.18*
Acetate	1,432.71±180.58	289.93±26.32*
Glycerol :		
Glucose	218.00±9.78	167.00±8.22*
Acetate	896.54±56.00	218.93±8.63*
Lipolysis (μM/h) :		
NEFA	71.09±55.58	1232.61±72.15

Means SE (n=15).
* : Means in the same row are significantly different ($p<0.05$).

Fasting reduced lipogenesis and increased the lipolysis rate both in growing and laying periods of ducks. Our previous experiments also indicated that liver lipogenic enzymes activities depressed in ducks as subjected to fasting, and the activities of the fatty acids β-oxidation related enzymes were markedly enhanced. This was also true for plasma NEFA and glycerol level. Whereas plasma VLDL and TG concentration significantly declined; abdominal fat obviously reduced as well (unpublished data).

Plasma glucagon concentration can be increased and glycolysis rate can be inhibited by fasting (Tanaka et al., 1984; Louis and Witters, 1992). Thus, acetyl-CoA production is reduced and lipogenesis rate is depressed consequently. Alternately, glucagon can stimulate lipolysis, particularly in poultry adipocytes. Their hormone-sensitive lipase is highly sensitive to glucagon and is approximately 10 fold that in mammalian's (Oscar, 1995). Therefore, fasting can increase the adipocytes lipolysis rate (Calabotta et al., 1985). Many investigations also confer that fasting can depress liver lipogenesis in vitro (Yeh and Leveille, 1971; Hasegawa et al., 1982; Calabotta et al., 1985). Our results presented herein were well correlated with above finding. Our recent study (unpublished data) and many reports also confer that fasting significantly decreases lipogenic enzymes activities of a ducks liver (Leveille et al., 1975; Hasegawa et al., 1982; Calabotta et al., 1985; Goldman et al., 1985).

Yen and Leveille (1970) have found that increase of the plasma NEFA level could depress lipogenesis. Our recent study has verified that fasting elevates plasma NEFA (Lien et al., 1999). NEFA would combine with CoA to form fatty acyl-CoA to proceed with the β-oxidation. Since lipogenesis is preceded by NEFA β-oxidation, an enhanced plasma NEFA was in competition for utilization of CoA with lipogenesis, Thus, available CoA was insufficient for lipogenesis. In addition, CoA participated citrate cleavage reaction to form acetyl-CoA and oxaloacetate. Notably, an elevation of plasma NEFA content and absence of free CoA was leading to a situation in which acetyl-CoA required for lipogenesis is reduced. Moreover, long chain fatty acyl-CoA depressed citrate transfer through mitochondria membrane into cytosol, which is a lipogenesis substrate and an acetyl-CoA carboxylase (ACC) activator. Therefore, an increased long chain fatty acyl-CoA content depresses ACC activity (Leveille and Yeh, 1975; Tanaka et al., 1979; Severson and Hurley, 1984), ultimately depresses lipogenesis (Yeh and Leveille, 1970).

In conclusion, this study demonstrates that the capacity of Taiwan Tsaiya ducks hepatocytes lipogenesis during laying period is greater than that during growing period. The ability of ducks in use of acetate for lipogenesis was higher than that is use of glucose.

In addition, the hepotocytes lipogenesis capacity were reduced and the adipocytes lipolysis rate were increased significantly by fasting.

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