Enzyme Activities Related to Lipid Metabolism in the Liver and Adipose Tissue of Tsaiya Ducks under Fasting and *Ad libitum* Feeding Conditions

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ABSTRACT : The study investigated the lipid metabolism of Tsaiya ducks under fasting and *ad libitum* feeding conditions. Sixty Tsaiya ducks in their growing period (8-12 wk-old) and sixty Tsaiya ducks in their laying period (26-30 wk-old, 10-14 weeks after the onset of laying) were randomly divided into *ad libitum* feeding and 3-day fasting groups. The activities of lipid metabolism related enzymes were determined. Experimental results indicated that fasting depressed the activities of lipogenesis related enzymes such as fatty acid synthetase and NADP-malic dehydrogenase in both periods ($p<0.05$). Fasting also increased the activities of liver fatty acid β-oxidation enzymes ($p<0.05$). However, the activities of lipoprotein lipase in adipose tissue, heart and ovarian follicle in both periods and the hormone-sensitive lipase of adipose tissue in the growing period were decreased by fasting ($p<0.01$). ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 403-408)***

Key Words : Tsaiya Duck, Enzyme Activities, Lipogenesis, Fatty Acid β-oxidation, Lipoprotein Lipase, Hormone-sensitive Lipase, Liver, Adipose Tissue

INTRODUCTION

Production of ducks is one of the leading avian industries in Taiwan. The lipid metabolism data of chickens are frequently used as the reference for ducks because the lipid metabolism of ducks has seldom been studied. However, the lipid metabolism of ducks as in waterfowl may differ from that of chickens. Bogin et al. (1984) and Avidar et al. (1986) indicated that the activities of the lipid metabolism related enzymes of geese (water fowl) differed from those of chickens while the geese were subjected to force feeding. Moreover, a certain kind of duck (such as mule duck) can be used for Foie Gras production (Lee et al., 1985, 1987), while chicken cannot be used for this purpose. In addition, the body fat of ducks is primarily located subcutaneously whereas in chickens it is located more in the abdominal region. The relative body fat of meat type ducks is approximately twice that of broiler chickens. The preceding distinctions imply that the lipid metabolism of these two types of avian species may be different.

Previous investigations have indicated that fasting decreases the lipogenesis capacity and increases the lipolysis rate of chickens (Calabotta et al., 1985; Goldman et al., 1985; Hasegawa et al., 1994b; Leveille et al., 1975). Other works noted increased activities of lipoprotein lipase (Hasegawa et al., 1994a) and hormone-sensitive lipase (Anthony et al., 1990) in fasting chickens. Also, the lipid metabolism of chickens in the laying period differs from that in the growing period (Walzem, 1996). In light of the preceding developments, this study elucidates the lipid metabolism in ducks by closely examining the activities of the lipid metabolism related enzymes of Tsaiya ducks in both growth and laying periods, under fasting and *ad libitum* feeding conditions.

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MATERIALS AND METHODS

Birds and treatment

The trial was performed with 120 layer Tsaiya ducks (*Anas platyrhynchos var. domestica*). Sixty ducks were in the growing period (8 weeks old), and sixty ducks were in the first laying period (26 weeks old or 10 weeks from the onset of laying). These ducks were randomly allocated to *ad libitum* feeding and 3 day fasting groups. The fasting group was continuously fasted for 3 days before the termination of the trial (12 weeks old and 30 weeks old, respectively). Table 1 lists the experimental diets in each period. Ducks were individually caged with unlimited access to water. The experiment lasted for 30 days in each period. Finally, the ducks were sacrificed and fresh liver, adipose tissue, thigh muscle, and follicle samples were taken from each duck and were put in an ice-cold 0.154 mM saline buffer (pH 7.2) to determining the activities of lipid metabolism related enzymes.

Sample preparation

Approximately 5 g of fresh liver was homogenized (Ystral, D-7801, Germany) with 15 ml of ice-cold 0.25 M sucrose and 5 M of Tris-HCl buffer (pH 7.2), the mixture was then centrifuged at 800×g for 10 min to remove the debris. The supernatant fraction was centrifuged at 15,000×g for 10 min to obtain mitochondria. The

Table 1. Composition of experimental diets (%)

Ingredients	Growing period	Laying period
Yellow corn meal	64.94	58.72
Soybean meal, 44%CP	16.43	27.38
Fish meal, 65%CP	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	0.03	0.03
Mineral premix	0.10	0.10
Soybean oil	-	1.23
Oyster shells	-	4.00
DL-Methionine	0.10	0.17
Choline chloride, 50%	1.00	0.59
Total	100.00	100.00
Calculated value		
Crude protein, %	16.00	19.00
ME, kcal kg^{-1}	2,730	2,730
Calcium, %	1.10	3.00
Available	0.60	0.45
Phosphorus, %		
Lysine, %	0.90	1.06
Methionine+Cystine, %	0.60	0.75
Choline, mg kg^{-1}	1,430	1,420

(1) Vitamin premix supplied the following per kilogram of diet: Vitamin A, 25,000 IU; Vitamin D_3, 3,125 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.

(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 (Zn, 80.35% Zn) 60 mg; Se ($NaSeO_3$, 45.56% Se) 0.075 mg.

supernatant was centrifuged at 105,000×g for 60 min (SW 41 rotor) with an ultracentrifuge (Beckman, Model L-8, USA), and the supernatant (cytosol fraction) was taken. Roughly 1 g of fresh adipose tissue, heart, follicle, or thigh muscle samples were homogenized in 10 ml of ice-cold 0.25 M sucrose and 1mM of EDTA-2Na buffer (pH 7.4), and were then centrifuged at 12,000×g for 30 min. The clear fraction was taken for determination of lipoprotein lipase or hormone-sensitive lipase activity.

All procedures were performed at 0-4°C and all samples were stored below -70°C until the enzyme activities were measured.

Determination of enzyme activity

To determine lipogenesis related enzyme activity, the liver cytosol samples were added to the incubation medium and measured by a spectrometer (Hitachi, U2000, Japan) at 340 nm in 25°C for 5 min. Acetyl-CoA carboxylase (EC 6.4.1.2, ACC) was determined according to the method of Numa (1969), to measure the rate of NADPH oxidized as the acetyl-CoA carboxylation took place. ATP-citrate cleavage enzyme (EC 4.1.3.8, CCE) was analyzed based on examining the rate of NADH oxidized following the methods described by Takada et al. (1963). NADP-malic dehydrogenase (EC 1.1.1.40, MDH) was determined on the rate of NADPH production as the reaction took place, 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) to determine the rate of NADPH oxidized during incubation. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49, G-6-PDH) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44, 6-GPDH) were determined following the method of Lohr and Waller (1974) to determine the NADPH production per minute as the reaction took place.

To determine the activities of fatty acid β-oxidation enzymes, the liver mitochondria samples were incubated in the medium, and measured at 25°C for 3 min. Carnitine palmitoyl transferase (EC 2.3.1.23, CPT) was analyzed using the method proposed by Bieber and Helmrath (1972), and the assay was based on measuring the initial CoASH formation by the NADPH, reacted on palmitoyl CoA by mitochondria samples with L-carnitine, and the reaction was monitored at 412 nm. Acyl-CoA dehydrogenase (EC 1.3.99.2, ACD) was measured by the rate of butyryl-CoA conversion to indophenol at 600 nm following the Hoskins (1969) procedure. Enoyl-CoA hydratase (EC 4.2.1.17, ECH) was measured by observing the reaction of hydration of crotonyl-CoA at 280 nm using the method described by Bradshaw and Noyes (1975). β-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35, HCD) was measured by the rate of NADH oxidation when the reaction took place, according to the method described by Bradshaw and Noyes (1975); β-ketothiolase (EC 2.3.1.16, KT) was measured by the reaction of acetoacetate cleavage, following the method of Middleton (1975).

Hormone-sensitive lipase (EC 3.1.1.3, HSL) was assayed according to the method proposed by Hasegawa et al. (1980a). In brief, minced adipose tissue sample was homogenized (Ystral, D-7801, Germany) in 6 volumes of 0.25 M sucrose (pH 6.8) containing 1 mM EDTA, then centrifuged at 12,000×g for 30 min in 0°C. The fat cake of the upper phase was carefully removed and the supernatant fluid of the medium fraction was used to assay activities. The HSL activity was determined by measuring the amount of glycerol released from hydrolysis of an artificial triacylglycerol emulsion.

Lipoprotein lipase (EC 3.1.1.34, LPL) was measured according to the procedure of Griffin et al. (1987). Adipose tissue sample was prepared to extract protein. The protein pellet was then extracted by 7.5 ml of acetone and once with 7.5 ml diethyl ether, both at 0°C. The LPL activity in extracts of the acetone-ether powders was assayed using isolated ducks chylomicron as substrate and activated by incubating for 1 h at 37°C with immature duck serum (3 ml

serum/mg chylomicron). Aceton-ether powders were added in 3 ml of 20 mM Tris-HCl (pH 8.0) containing 20 μg heparin/ml. Then, duplicate of 100 μl aliquots of extract were incubated with 10 μl activated chylomicron in 250 μl of 50 mM Tris-HCl (pH 8.0) containing 20 g bovine serum albumin/L. Reactions were stopped after 1 h at 37°C by the addition 2.4 ml of 0.1 M glycine buffer (pH 2.7). Glycerol was then measured to express LPL activities, using the method of Imai (1967).

Liver cytosol and mitochondria protein were measured employing the biuret method (Robson and Goll, 1968). Adipose tissue, heart, follicle, and thigh muscle protein were assayed using the Lowry method (Lowry et al., 1951).

Statistical analyses

All data were statistically analyzed using SAS software and analyses of variance. The significance was determined using Tukey's test (SAS, 1990).

RESULTS

Table 2 lists the activities of lipid metabolism related enzymes of Tsaiya ducks during the growing period (8-12 wk old). The activities of lipogenesis related enzymes were decreased by fasting ($p<0.01$) with the exception of 6-PGDH. The activities of liver fatty acid β-oxidation enzymes except ECH, were significantly increased by fasting ($p<0.001$). LPL activities in adipose tissue and heart, and the activity of hormone-sensitive lipase in adipose tissue were markedly depressed by fasting ($p<0.01$). However, LPL activity in the thigh muscle was not affected ($p>0.05$).

Table 3 presents the activities of lipid metabolism related enzymes during the laying period. This table reveals that the activities of lipogenesis enzymes in laying ducks were higher than those in growing ducks. The activities of most lipogenesis enzymes except ACC were depressed by fasting ($p<0.05$). In addition, the activities of liver fatty acid β-oxidation enzymes except HCD, were significantly increased by fasting ($p<0.05$). LPL activities of adipose tissue and heart in laying ducks were higher than in growing ducks. Moreover, LPL activity in various organs was markedly diminished by fasting ($p<0.001$). However, the activity of adipose tissue HSL was not influenced by fasting ($p>0.05$).

DISCUSSION

This study investigated the lipid metabolism of Tsaiya ducks under fasting and *ad libitum* feeding conditions. When birds are laying, the ovarian follicles secrete estrogen to stimulate liver lipogenesis and to depress the rate of fatty acid β-oxidation. This event obviously enhances plasma TG (Hasegawa et al., 1980b; Leclercq, 1984). This study demonstrated that the activities of liver lipogenesis enzymes of ducks are higher during the laying period than during the

Table 2. The activities of lipid metabolism related enzymes of Tsaiya ducks during growing period (8-12 wk old)

Items	Fed	Fast
Lipogenesis:		
Acetyl-CoA carboxylase (nmole mg protein^{-1} min^{-1})	1.38±0.24	0.83±0.08**
Fatty acid synthetase (nmole mg protein^{-1} min^{-1})	7.10±0.53	3.90±0.47***
ATP-Citrate cleavage enzyme (nmole mg protein^{-1} min^{-1})	57.61±1.91	34.17±3.07***
NADP-Malic dehydrogenase (nmole mg protein^{-1} min^{-1})	318.94±28.04	52.87±13.03***
Glucose-6-phosphate dehydrogenase (nmole mg protein^{-1} min^{-1})	3.85±0.36	1.60±0.18***
6-Phosphogluconate dehydrogenase (nmole mg protein^{-1} min^{-1})	2.44±0.34	2.46±0.28
Fatty acid β-oxidation:		
Carnitine palmitoyl transferase (nmole L-carnitine exchanged mg protein^{-1} min^{-1})	17.79±1.93	33.46±2.06***
Enoyl-CoA hydratase (unit mg protein^{-1} min^{-1})[a]	128.68±6.55	146.28±12.57
Acyl-CoA dehydrogenase (unit mg protein^{-1} min^{-1})[b]	1,815.82±251.20	10,687.04±706.82***
β-hydroxyacyl-CoA dehydrogenase (unit mg protein^{-1} min^{-1})[c]	0.62±0.10	5.22±0.42***
β-Ketothiolase (unit mg protein^{-1} min^{-1})[d]	0.94±0.12	1.99±0.06***
TG mobilization:		
Lipoprotein lipase of heart (μmole glycerol released mg protein^{-1} h^{-1})	28.21±2.83	20.05±0.88**
Lipoprotein lipase of thigh muscle (μmole glycerol released mg protein^{-1} h^{-1})	22.36±1.83	19.65±1.19
Lipoprotein lipase of adipose tissue (μmole glycerol released mg protein^{-1} h^{-1})	32.72±3.01	19.76±1.04***
Hormone sensitive lipase of adipose tissue (μmole glycerol released mg protein^{-1} h^{-1})	29.97±0.85	20.90±1.57***

Means±SE (n=30).

** $p<0.01$. *** $p<0.001$.

[a] 1 unit of enoyl-CoA hydratase activity is ABS decreased 0.01/min or hydration of 0.0045 μmole of crotonyl-CoA.

[b] 1 unit of acyl-CoA dehydrogenase activity is ABS decreased 0.001/min or reduction 1 μmole of indophenol.

[c] 1 unit of β-hydroxyacyl-CoA dehydrogenase activity is ABS decreased 0.001/min or oxidized 1μmole of NADH.

[d] 1 unit of β-ketothiolase activity is ABS decreased 0.01/min or 1 nmole of acetoacetate cleavaged.

Table 3. The activities of lipid metabolism related enzymes of Tsaiya ducks during laying period (26-30 wk-old, 10-14 weeks after the onset of laying)

Items	Fed	Fast
Lipogenesis:		
Acetyl-CoA carboxylase (nmole mg protein^{-1} min^{-1})	1.41±0.15	1.08±0.08
Fatty acid synthetase (nmole mg protein^{-1} min^{-1})	11.19±0.90	6.47±0.85***
ATP-citrate cleavage enzyme (nmole mg protein^{-1} min^{-1})	76.52±3.67	62.74±2.76*
NADP-Malic dehydrogenase (nmole mg protein^{-1} min^{-1})	557.42±26.54	159.65±28.46***
Glucose-6-phosphate dehydrogenase (nmole mg protein^{-1} min^{-1})	22.74±1.88	18.18±1.10*
6-phosphogluconate dehydrogenase (nmole mg protein^{-1} min^{-1})	20.66±1.19	15.33±1.94*
Fatty acid β-oxidation:		
Carnitine palmitoyl transferase (nmole L-carnitine exchanged mg protein^{-1} min^{-1})	13.08±2.40	25.60±2.76**
Enoyl-CoA hydratase (unit mg protein^{-1} min^{-1})	22.85±1.52	62.45±4.83***
Acyl-CoA dehydrogenase (unit mg protein^{-1} min^{-1})	1,499.31±225.08	2,369.34±346.94*
β-hydroxyacyl-CoA dehydrogenase (unit mg protein^{-1} min^{-1})	1.52±0.19	2.17±0.30
β-ketothiolase (unit mg protein^{-1} min^{-1})	2.06±0.09	2.34±0.10*
TG mobilization:		
Lipoprotein lipase of heart (μmole glycerol released mg protein^{-1} h^{-1})	18.33±1.25	9.96±0.91***
Lipoprotein lipase of follicle (μmole glycerol released mg protein^{-1} h^{-1})	16.41±1.14	11.04±1.28**
Lipoprotein lipase of adipose tissue (μmole glycerol released mg protein^{-1} h^{-1})	19.11±1.12	11.23±1.43***
Hormone sensitive lipase of adipose tissue (μmole glycerol released mg protein^{-1} h^{-1})	29.91±1.97	27.96±1.47

Means±SE (n=30).
* $p<0.05$. ** $p<0.01$. *** $p<0.001$.

growing period. The activities of liver fatty acid β-oxidation enzymes are lower during the laying period than during the growing period. Consequently, plasma TG levels of ducks and hens during the laying period were higher than during the growing period, is in agreement with our previous study (1,456.43 vs 223.63 mg dl^{-1}), (Lien et al., 1999, 2001). A bird during the laying period, or after injection of estrogen, would have depressed LPL activity and an increased plasma VLDL level, and a slow clearance of plasma TG (Hasegawa, 1980a; Tanaka et al., 1986).

Previous investigations have indicated that fasting depresses enzyme activities related to lipogenesis (Calabotta et al., 1985; Goldman et al., 1985; Hasegawa et al., 1994b; Leveille et al., 1975). Yeh and Leveille (1970) reported that fasting markedly increasd plasma NEFA in chickens. As NEFA is metabolized via β-oxidation, it combines with CoA to form fatty acyl-CoA; thus available CoA is insufficient for lipogenesis. In addition, investigators have observed that increased long chain fatty acyl-CoA depresses ACC activity, because long chain fatty acyl-CoA depresses the transfer of citrate through the mitochondria membrane into cytosol and citrate is an ACC activator and the substrate of lipogenesis (Leveille et al., 1975; Severson and Hurley, 1984; Takada et al., 1963). Consequently, lipogenesis is depressed (Yeh and Leveille, 1970).

ACC is a rate-limiting enzyme of lipogenesis. During feeding, ACC activity increases and malonyl-CoA level increases. Additionally, fatty acid β-oxidation is suppressed because malonyl-CoA inhibits the activity of CPT (β-oxidation rate limiting enzyme) through allosteric regulation (Bird et al., 1985; McGarry and Foster, 1979; McGarry et al., 1978a; 1978b; 1978c). Consequently, fasting depresses ACC activity; malonyl-CoA level is lowered, CPT is not inhibited, and then β-fatty acid oxidation is facilitated. The results of this study also demonstrate that fasting increases CPT activity and other liver fatty acid oxidation enzyme activities.

Yeh and Leveille (1970) indicated that fasting depressed the activity of NADPH producting enzymes, such as MDH. This event may result from fatty acyl-CoA inhibiting citrate transfer, as fasting increases the plasma NEFA level. Consequently, malate formation is depressed, leading to diminished activity of MDH. The activities of G-6-PDH and 6-PGDH are reduced as well.

Freedland (1967) demonstrated that the activities of liver lipogenesis associated enzymes of rats significantly decreased when subjected to progressive starvation. However, the enzyme activities involved in gluconeogenesis and the TCA cycle was equally stable toward starvation. Goodridge (1968) also indicated that the activities of MDH and CCE were at an extremely low stability in chick embryonic liver. However, they increased 15-85 fold by the time the chicks at 5-7 days old under *ad libitum* feeding. Gluconeogenesis is important for energy production to maintain life. Thus, under starvation circumstances, the NADH/NAD$^+$ and NADPH/NADP$^+$ ratios decreased, and NADPH eventually becomes insufficient for lipogenesis. This study confirms that fasting decreases the NADPH production related enzyme activities; the activities of lipogenesis-related enzymes are also

reduced. This occurrence corresponds to the observation in this study that lipogenesis is an energy balance dependent process.

The results of Hasegawa et al. (1994a) indicated that the LPL activity of chicks decreased during fasting. Also, Gasquet and Pequignot (1972) noted that LPL activity decreased in mouse adipose tissue during fasting. In pigs, however, LPL activity in the heart increases, while fasting decreases adipose tissue LPL (Enser, 1973). These conflicting observations may be attributable to the differences of LPL property among the different species of animals (Sato et al., 1994; Benson and Bensadoun, 1977).

According to Anthony et al. (1990), fasting increases plasma NEFA in turkeys, probably through enhanced HSL activity, but this does not agree with observation in this study. This discrepancy may be due to the difference in expressing the results. In this study, data are expressed as μmole mg protein^{-1} h^{-1}, which is based on cellular protein. If expressed as wet weight, as in Anthony et al. (1990), fasting appears to enhance HSL. When an equal weight of adipose tissue is taken from fasted animals, the adipocyte volume decreases and the adipocyte number increases as well. Moreover, turkeys are very particularly lean animals (turkey never contain more than 12% of fat in their body) compared to waterfowl in general, and their lipid metabolism could consequently be very different. Severson and Hurly (1984) suggested that fasting not only elevates the plasma NEFA, but also enhances the fatty acyl-CoA to inhibit HSL activity. This occurrence may be a feedback mechanism to inhibit lipolysis. The feedback mechanism may result from NEFA inhibiting on cAMP by prohibiting the adenylate cylase activity. Therefore HSL may be depressed by fasting, as evidenced in this study.

CONCLUSION

This study investigated the lipid metabolism of Tsaiya ducks under fasting and *ad libitum* feeding conditions. According to our results, fasting not only reduced the enzyme activities associated with liver lipogenesis and lipoprotein-TG degradation, but also increased the activities of liver fatty acid β-oxidation enzymes. In addition, the lipogenesis capacity in laying ducks was higher than that in growing ducks, while the fatty acid β-oxidation and lipoprotein-TG degradation activities in laying ducks were lower than in growing ducks.

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