Effects of Different Fatty Acids and Levels on the Lipogenesis Capacity and Lipolysis Rate of Broilers *In Vitro*

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ABSTRACT : This study investigated the lipogenesis capacity of hepatocytes and lipolysis rate of adipocytes of broilers as affected by different fatty acids (trial one) and different linoleic acid (C18:2) levels (trial two). Twenty 6-wk old broilers were used; their hepatocytes and adipocytes were isolated for the *in vitro* study. In trial one, four treatments were tested. The control group in which no fatty acid was added, and the test groups to which were added 300 μM of C16:0, C18:1 and C18:2, respectively. For trial two, different levels (0, 300 μM and 1 mM) of C18:2 combined to fatty acid-free bovine serum albumin (BSA) were added to the medium. According to results of trial one, added fatty acids significantly reduced the incorporation by hepatocytes of [U,^{14}C]glucose into total lipid ($p<0.05$); the lipogenesis capacity in C18:2 group was the lowest. Although a similar pattern was found with [1,^{14}C]acetate, the groups only slightly differed in terms of lipogenesis capacity ($p=0.11$). In addition, the C18:2 group had a significantly ($p<0.05$) greater lipolysis rate than the C16:0 and control groups. Results of trial two indicated that C18:2 significantly ($p<0.05$) reduced lipogenesis capacity both for [U,^{14}C]glucose and [1,^{14}C]acetate, and markedly stimulated the lipolysis rate ($p<0.05$), displaying a dose response. Results presented herein demonstrate that C18:2 can reduce lipogenesis capacity and stimulate the lipolysis rate in broilers. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 9 : 1285-1289*)

Key Words : Fatty Acids, Lipogenesis, Lipolysis

INTRODUCTION

Broilers grow rapidly and always have much abdominal fat when they are marketed. Abdominal fat is the low value part, therefore many approaches have been developed to reduce the amount in the birds. Among these methods is the use of different dietary fat sources, but their use has yielded markedly different results.

Lien et al. (1994) indicated that broilers fed a diet supplemented with soybean oil had less abdominal fat and liver fat content than those fed a diet supplemented with full fat peanut meal (enriched in C18:1). Hepatic lipogenic-related enzyme activities were also lower in soybean oil supplemented broilers. Later, Li (1995) observed a similar phenomenon, indicating that a diet supplemented with different dietary fat sources could affect geese abdominal fat weight: in the lard (abundant in C16:0 and C18:1) supplemented group more abdominal fat was produced than in the soybean oil supplemented group (abundant C18:2). Plasma phospholipid and nonesterified fatty acid (NEFA) concentration in the soybean oil fed group was significantly higher than in the geese fed a control diet. The activities of hepatic lipogenic-related enzymes were lower than in the control group as well. Moreover, Iritani and Fukuda (1980) reported that feeding rats a diet containing 10% corn oil (abundant C18:1 and C18:2) for 2 weeks, reduced the activities of lipogenic and glycerol lipid synthesis enzymes. Hillard et al. (1980) indicated that supplementing chicken diets with unsaturated fatty acids could affect lipogenesis capacity, and Keren-Zvi et al. (1990) reported that supplementing broiler diets with soybean oil could reduce their abdominal fat content. But there are also reports that dietary unsaturated fatty acids could not reduce a bird's body fat (Hulan et al., 1984; Olomu and Baracos, 1991) thus this subject requires further investigation. As is generally known, the *de novo* lipogenesis in birds is found primarily in the liver. Therefore, this study investigated the effect of different fatty acids and fatty acid levels on broiler hepatocyte lipogenesis capacity and adipocyte lipolysis rate by employing an *in vitro* method.

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MATERIALS AND METHODS

Animal treatment

Twenty 6 week-old broilers fed the same diets (CP 23.07 and 20.26%, ME 3214 and 3217 kcal/kg in 0-3 weeks and 3-6 weeks, respectively) and housed in the same environment (average temperature 31.26±2.27℃ in the day time) were used to investigate how different fatty acids (FA) and different linoleic acid (C18:2) levels influenced hepatocyte lipogenesis capacity and adipocyte lipolysis rate *in vitro*. In trial one, different fatty acids (C16:0, C18:1 and C18:2) combined with fatty acid-free bovine serum albumin (since in blood it is present as the fatty acid-albumin

form) were added to the control medium (no fatty acids) at a level of 300 μM. In trial two, different levels of linoleic acid were used at 0, 300 μM and 1 mM to measure the effect on lipogenesis capacity and lipolysis rate.

Isolation of hepatocytes

Broilers were anesthetized with sodium barbital (130 mg/100 ml) (50 ml/kg BW), and then disinfected with iodine and 70% alcohol. The abdomen was opened using a pair of scissors. The liver was then infused with 40℃ HBSS (Hanks balanced salt solution) buffer (pH 7.4) (added 2 ml 50 mM EGTA/200 ml buffer) from the portal vein using a pump (40 ml/min). The liver surface was massaged to facilitate flushing 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 HBSS buffer solution (10-20 ml/min) containing 70 mg collagenase (type IV)/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. The liver was then removed and scraped with a No. 22 needle. The suspension solution was filtered through 250 and 100 mesh nylon screens. The hepatocytes were then washed three times with a HBSS buffer solution, centrifuged at 50 g for 3 min and a DMEM (Dulbeccos modified Eagles 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. The cells were then counted using the red blood cell examination method (Schultz and Mistry, 1981).

Isolation of adipocytes

The fresh broiler abdominal fat tissue was removed and placed in polypropylene Erlenmeyer flasks. After the addition of 1.5 ml of KRB-HEPES (Krebs-Ringer-bicarbonate-N-[2-hydroxy-ethyl]piperazine-N-[2-ethane sulfonic acid]) buffer containing 3 mg collagenase (type I) per g of tissue, the adipose tissue was cut into finely minced pieces using a pair of scissors. The pieces were then incubated with a shaker (90 strokes/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 using hematocrit examination method (Novakofski and Hu, 1987).

Determination of lipogenesis capacity

The hepatocytes (1×10^6 cells) suspended in a 5 ml KRB-HEPES buffer (which contained 20 mM glucose), were added to a medium containing 0.5 μCi[U,^{14}C] glucose or 1 μCi[1,^{14}C]acetate, respectively. Incubation was performed at 37℃, in an atmosphere supplied with 95% O_2 and 5% CO_2 and shaken at 90 strokes/min for 3 h. Next, 0.25 ml of 1 N H_2SO_4 was then added to terminate the reaction. Total lipids were extracted according to the method of Folch et al. (1957). After evaporating the solvent, 10 ml of scintillation solution (per litter contain 385 ml perdioxane, 385 ml xylene, 230 ml ethanol, 5 g 2,5-diphenyloxazole, 0.05 g α-naphthyl-phenyloxagole and 80 g naphthalene) was added, and the radio-activity was determined using a liquid scintillation counter (Beckman, LS3801, USA) (Mersmann and Hu, 1987). Two replicates per bird were tested.

Determination of lipolysis rate

The adipocytes (1×10^6 cells) were added in a 5 ml KRB medium containing 4% fatty acid-free bovine serum albumin. The mixture was incubated at 37℃, supplied with 95% O_2 and 5% CO_2 air and shaken at 90 strokes/min for 3 h. Then the incubated tube were placed into ice cold water to terminate the reaction (Mersmann and Hu, 1987). The NEFA concentration was measured according to Chromy et al. (1977) to determine the lipolysis rate. The FA that was added to the medium was deducted from the total FA. Two replicates per bird were tested.

Statistical analysis

SAS software was used to analyze the variance. The significant difference was then determined with Tukey's test (SAS, 1990).

RESULTS AND DISCUSSION

Table 1 summarizes how different fatty acids influence the lipogenesis capacity of the hepatocytes and adipocytes lipolysis rate of the broilers. According to these data, adding fatty acids to the medium significantly ($p<0.05$) reduced hepatocytes incorporation of [U,^{14}C]glucose into total lipid. Although the fatty acid groups did not significantly differ, C18:2 was the lowest. Notably, a similar pattern was also found in [1,^{14}C]acetate, even though there was no statistically significant difference ($p=0.11$). As for the lipolysis rate, C18:2 had a significantly higher rate than C16:0 and the control ($p<0.05$).

In related work, Iritani and Fukuda (1980) indicated that corn oil feeding reduced the incorporation of labeled glycerol or palmitic acid into the triacylglycerol (TG) of the liver slices. Grundy (1987) compared different fatty acids (C16:0, C18:1 and C18:2) on human plasma TG levels, indicating that the effect is C18:2<C18:1<C16:0. This observation suggests that lipogenesis of the C18:2 group was less than the C18:1 and C16:0 groups. Toussant et al. (1981) and Hillard et al. (1980) indicated that

Table 1. Effects of different fatty acids on the lipogenesis of hepatocyte and lipolysis of adipocyte capacity of broilers *in vitro*

	Fatty acid medium			
	Control	C16:0	C18:1	C18:2
Lipogenesis:				
Glucose, dpm min^{-1} ml^{-1}	1035± 92[a]	750± 80[b]	623± 42[b]	522± 45[b]
Acetate, dpm min^{-1} ml^{-1}	6471±511	6271±503	5398±481	5015±437
Lipolysis:				
NEFA, μM	242± 14[b]	233± 12[b]	250± 11[ab]	297± 14[a]

Means±SE (n=40).

[a,b] Means in the same row with no common superscript are significantly different ($p<0.05$).

lipogenesis depression by unsaturated fatty acids was greater than by saturated fatty acids. In addition, the dietary saturated fatty acids had the tendency to be stored as body fat (Van Es, 1977; Deaton et al., 1981; Awad et al., 1990), whereas dietary unsaturated fatty acids were used primarily for β-oxidation. And Keren-Zvi et al. (1990) and Li (1995) demonstrated that birds fed a diet containing soybean oil had reduced abdominal fat. The above investigations correspond to the results in this study.

Table 2 presents the different levels of C18:2 on the lipogenesis capacity of hepatocytes and the adipocyte lipolysis rate of broilers. This table reveals that adding C18:2 significantly ($p<0.05$) reduced hepatocyte lipogenesis capacity both in [U,^{14}C]glucose and [1,^{14}C]acetate medium. There was also a dose response in the [1,^{14}C]acetate treatment. This result further indicates that adding C18:2 can markedly ($p<0.05$) stimulate the adipocytes lipolysis rate.

Yen and Leveille (1970) and Donaldson (1985) reported that the hepatic lipogenesis capacity of birds was reduced when serum nonesterified fatty acid (NEFA) concentration was increased. NEFA would combine with CoA to form fatty acyl-CoA to proceed with the β-oxidation. Since lipogenesis is preceded by NEFA β-oxidation, 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 the citrate transfer through the 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 et al., 1975; Tanaka et al., 1979; Severson and Hurley, 1984), and ultimately depresses lipogenesis (Yeh and Leveille, 1970).

Heemskerk et al. (1995) reported that dietary fatty acids could alter the membrane cholesterol/phospholipid ratio and signal transduction. In general, the saturated-fat diet results in less responsiveness to the agonist. Neelands and Clandinin (1983) indicated that increasing the level of dietary C18:1 would increase the level of membrane C18:1 and total mono-unsaturated fatty acids in phosphatidylcholine and phosphatidylethanolamine. Such an increase is associated with a corresponding increase in glucagon-stimulated adenylated cyclase activity and elevated intracellular cAMP formation (Morson and Clandinin, 1983). As is generally recognized, chicken hormone-sensitive lipase (HSL) in intracellular adipocytes is highly sensitive to glucagon. Therefore, glucagon apparently stimulated lipolysis in primary cultured broiler adipocytes (Oscar, 1991). This mechanism corresponds to our finding in which C18:2 could stimulate the lipolysis rate of adipocytes *in vitro*.

Iritani et al. (1995) reported that feeding a hydrogenated fat diet markedly increased the hepatic mRNA concentrations and lipogenic enzyme activities. However, those hepatic mRNA concentration and lipogenic enzyme activities were markedly reduced as the diet was changed to one containing corn oil. Concurrent insulin binding to receptors in the liver was reduced by the corn oil diet (abundant in C18:1 and C18:2). Notably, insulin could stimulate the glucose transport and metabolism, to supply

Table 2. Effects of three levels of C18:2 on the lipogenesis of hepatocytes and lipolysis of adipocytes capacity of broilers *in vitro*

	0	300	1000 (μM)
Lipogenesis:			
Glucose, dpm min^{-1} ml^{-1}	1035± 92[a]	522± 45[b]	444± 35[b]
Acetate, dpm min^{-1} ml^{-1}	6471±511[a]	5015±437[b]	3073±317[c]
Lipolysis:			
NEFA, μM	242± 14[b]	297± 14[a]	333± 10[a]

Means±SE (n=40).

[a,b] Means in the same row with no common superscript are significantly different ($p<0.05$).

acetyl-CoA and NADPH for fatty acid synthesis (Laurin and Cartwright, 1993). Increasing the level of pyruvate dehydrogenase (Denton and Halestrape, 1979), acetyl-CoA carboxylase (Brownsey et al., 1984), ATP-citrate lyase and malic enzyme (Brownsey et al., 1984; Laurin and Cartwright, 1993) can achieve this supply. These investigations support our findings that C18:1 and C18:2 can reduce lipogenesis capacity. Moreover, Montalto and Bensadoun (1993) indicated that in cultured avian adipocytes, administering C18:2 decreased lipoprotein lipase secretion, synthesis and level of mRNA. Thus, adipocytes fed C18:2 would be expected to deposit less fat than those supplied saturated fatty acids.

CONCLUSION

This study demonstrated that added C18:2 reduced hepatocyte lipogenesis and enhanced the adipocyte lipolysis rate. Therefore, the *in vitro* results support other *in vivo* studies in which supplementation with dietary fat rich in C18:2 can reduce body fat deposition.

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