Incorporation of [$1-^{14}\text{C}$] Sodium Acetate into the Fatty Acid Fraction of the Liver and Ovarian Follicles in Laying Japanese Quail (*Coturnix coturnix japonica*)

Kazumi KITA, Atsushi MURAI, Mitsuhiro FURUSE,
Koshi ASAKURA and Jun-ichi OKUMURA

School of Agriculture, Nagoya University, Chikusa-ku,
Nagoya-shi 464-01

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Abstract Time course change in the incorporation of [$1-^{14}\text{C}$] sodium acetate into the fatty acid fraction of the liver and ovarian follicles in laying Japanese quail (*Coturnix coturnix japonica*) were measured after intravenous injection of [$1-^{14}\text{C}$] sodium acetate. Exactly at 0.5, 1, 2, 4, and 6 hours after a single dose of the tracer, liver and ovarian follicles were excised and the radioactivity of ^{14}C incorporated into fatty acids in each tissue was determined. The incorporation of [$1-^{14}\text{C}$] sodium acetate into fatty acids in the liver after 0.5 hours of the injection was the highest of all, and it decreased significantly within the following 0.5 hours. In the ovarian follicles, on the contrary, the ^{14}C radioactivity in fatty acids started to increase gradually from 2 to 6 hours after a single injection of [$1-^{14}\text{C}$] sodium acetate. The lower ^{14}C radioactivity in ovarian follicles at the early time after injection would lead to the conclusion that little fatty acids are synthesized in the ovarian follicles of the laying Japanese quail, and there is the possibility that the progressive accumulation of fatty acids would be transported from other tissues to yolk in ovarian follicles.

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Key words : Japanese quail, fatty acid synthesis, liver, ovarian follicles

Although both liver and adipose tissue are the major sites of fatty acid synthesis in mammalian species, in the chicken, most of fatty acids are synthesized in the liver⁴⁾. Especially, O'Hea and Leveille⁵⁾ reported that between 90 to 95% of *de novo* fatty acid synthesis in the chick appeared to take place in the liver. In the laying hen, triglyceride-rich lipoprotein which includes fatty acids synthesized in hen body is transported to the ovary and transferred to the growing oocytes as major yolk precursors³⁾.

In the laying quail, the average egg weight is

approximately 10 g, which represents approximately 8% of body weight, and the yolk containing about 30-35% of lipid corresponds to approximately 35% of the egg weight⁶⁾. This fact indicates that the large amount of lipid corresponding 1% of body weight will be deposited in the ovarian follicles within about one day. Therefore, the laying quail needs to synthesize a large amount of fatty acids to supply lipids sufficiently into the ovarian follicles. However, there were few available studies *in vivo* investigating about fatty acid synthesis in the liver and fatty acid deposition

in ovarian follicles of the laying quail. In the present study, therefore, incorporation of [$1-^{14}\text{C}$] sodium acetate into the liver and ovarian follicles was measured to investigate fatty acid synthesis in the liver and fatty acid deposition in ovarian follicles of laying Japanese quail (*Coturnix coturnix japonica*).

Materials and Methods

Fifty female Japanese quail (*Coturnix coturnix japonica*) were purchased from a local hatchery (Chubu Quail Incubation Company. Ltd., Toyohashi-shi) when they were 30 days of age. All birds were kept in stainless wire cages with artificial lights on 0800–2200 hour in a temperature-controlled room at $25 \pm 2^\circ\text{C}$. A practical diet for quail (Toyohashi Feed Mill Co. Ltd., Toyohashi-shi) containing crude protein 23%, crude fat 3%, crude ash 14%, crude fiber 6% and carbohydrate 54% was given *ad libitum* with free access to fresh water. At 13 weeks old, all quail were weighed and twenty-five birds were distributed into five experimental groups of five birds each so that mean body weights were as uniform as possible. To measure the rate of lipid synthesis all quail were injected intravenously via wing vein with [$1-^{14}\text{C}$] sodium acetate (1 ml/100 g body weight, 2.04 GBq/mmol, 185 kBq/ml; Amersham Japan, Tokyo) in 0.85% (w/v) sodium chloride. Exactly at 0.5, 1, 2, 4, and 6 hour after a single injection of the tracer, each group of five quail was killed by cervical

dislocation and excised the liver and ovarian follicles. After rinsing with the saline and blotting, all tissues were weighed and frozen in liquid N_2 . Tissue samples were stored at -20°C until analysis. About 1 g of frozen liver and ovarian follicles were weighed accurately and total lipids were extracted by using chloroform-methanol (2 : 1 (v/v)) solvent²⁾. After evaporation to remove the organic solvent, lipid residue was saponified with 8 ml of 10% (w/v) methanolic potassium hydroxide at 80°C for 2 hours. After cooling down to the room temperature, non-saponifiable lipids were extracted three times in 3 ml of light petroleum ether (b.p. $30-60^\circ\text{C}$). Saponified samples were acidified with 3 ml of 9 M sulfuric acid, and then fatty acids were extracted three times in 3 ml of light petroleum ether. The radioactivity of ^{14}C in fatty acids was determined with the liquid-scintillation counter (LS 3801, Beckman Instrument Incorporation, CA, U.S.A.). The significance of difference between means of each parameter was assessed by a least significant difference test⁷⁾. All statistical analyses were done with a commercial statistical package, SAS (SAS Institute Inc., NC, U.S.A.).

Results and Discussion

Table 1 shows the weights of body, liver and ovarian follicles of laying Japanese quail. There were no significant differences in weights of body, liver and ovarian follicles among various time after the single injection of radio-

Table 1. Body weight, liver weight and ovarian follicles weight in Japanese quail (*Coturnix coturnix japonica*)

	Time after injection of [$1-^{14}\text{C}$] sodium acetate (hour)					Pooled SEM
	0.5	1	2	4	6	
Body weight (g)	132	132	133	133	132	4.2
Liver weight (g)	3.7	3.3	3.4	3.1	3.3	0.3
Ovarian follicles weight (g)	6.6	5.9	4.9	5.3	6.2	0.6

The number of birds used were five.

Means within the same row are not significantly different at $P < 0.05$.

Lipid Synthesis in Japanese Quail

Table 2. Time course change in the radioactivity and the recovery % of dose in fatty acids in the liver and ovarian follicles of Japanese quail (*Coturnix coturnix japonica*) injected with [1-¹⁴C] sodium acetate.

	Time after injection of [1- ¹⁴ C] sodium acetate (hour)					Pooled SEM
	0.5	1	2	4	6	
Liver						
Radioactivity (dpm × 10 ³)	216.6 ^a	72.1 ^b	89.2 ^b	67.2 ^b	76.5 ^b	30.9
Recovery % of dose	1.46 ^a	0.48 ^b	0.60 ^b	0.47 ^b	0.52 ^b	0.20
Ovarian follicles						
Radioactivity (dpm × 10 ³)	13.3 ^a	11.9 ^a	35.0 ^a	85.9 ^a	247.1 ^b	41.0
Recovery % of dose	0.09 ^a	0.08 ^a	0.23 ^a	0.60 ^a	1.70 ^b	0.29

The number of birds used were five.

Means not sharing a common superscript letter within the same row are significantly different at $P < 0.05$ (a and b).

All quail were injected intravenously via wing vein with [1-¹⁴C] sodium acetate (185 kBq/100g body weight).

active tracer.

Table 2 shows the time course of [1-¹⁴C] sodium acetate incorporation expressed as actual radioactivity and recovery percentage of dose into fatty acids of the liver and ovarian follicles in laying Japanese quail. According to the compartmental analysis⁸⁾, the absolute synthesis rate of the specific metabolite can be determined theoretically from the time course change in the specific radioactivity of the metabolite and its precursor after the tracer was given as a single dose. In the present study, however, only the radioactivity of ¹⁴C incorporated into fatty acids was determined and consequently the absolute rate of fatty acid synthesis was not calculated, because of the difficulty to measure the time course change in specific radioactivity of both objective fatty acid and its precursor. Therefore, in this study, the rate of fatty acid synthesis was described as the incorporation of [1-¹⁴C] sodium acetate into fatty acids in terms of 10³ dpm and as the recovery % of injected [1-¹⁴C] sodium acetate.

The incorporation of [1-¹⁴C] sodium acetate into fatty acids in the liver of laying Japanese quail at 0.5 hour after the injection was highest of all, and 1.46% of the dose was recovered

(Table 2). The large part of injected [1-¹⁴C] sodium acetate seemed to be expired in the form of CO₂, since it has been reported by TANAKA *et al.*^{9,10)} that the production of CO₂ from acetic acid was much more than that of fatty acids. The incorporated [1-¹⁴C] sodium acetate in the liver decreased rapidly within the subsequent 0.5 hours and remained almost constant after 1 hour of the injection, which is in good agreement with the result reported in young chickens⁵⁾. According to the findings by HIKAMI *et al.*¹¹⁾ and MIZUNO *et al.*¹²⁾, the recovery % of dose ranged from 2.15% to 6.06% at 10 min after injection of [1-¹⁴C] sodium acetate. Considering the time from 0.5 to 6 hours to measure the radioactivity incorporated into fatty acids, the value for the recovery % of dose in the present study were consistent with the previous findings. Although the tracer would reach simultaneously to liver and ovarian follicles with the intravenous injection of [1-¹⁴C] sodium acetate, in contrast to the prompt increase of ¹⁴C in the liver, the incorporation of [1-¹⁴C] sodium acetate into fatty acids in ovarian follicles was remained almost constant at the low level until 1 hour after the injection and increased exponentially until 6 hour after the single dose of tracer (Table 2).

According to the *in vitro* study reported by Weiss¹³⁾ in the laying hens, the ability of fatty acid synthesis in the ovarian tissue was much lower than that in the liver. Therefore, the lower ¹⁴C in ovarian follicles at the early time after the injection may lead to the speculation that little fatty acids are synthesized in the ovarian follicles of the laying Japanese quail similar to laying hens. In laying hens, Annison¹⁾ and GRIFFIN *et al.*³⁾ showed that precursors of egg yolk lipid including triglyceride-rich lipoprotein was synthesized in the liver, and in laying Japanese quail, there is the possibility to find out the similar transport mechanism of fatty acids from other tissues to yolk in overall follicles. However, to clarify the transport mechanism of fatty acids in detail, it is necessary to determine the time course change in the specific radioactivity of triglyceride-rich lipoprotein, which is the main precursors of egg yolk lipid in the liver, plasma, ovarian follicles and other tissues, and this remains to be studied the future.

References

- 1) ANNISON, E.F., Lipid metabolism. in Physiology and Biochemistry of the Domestic Fowl vol. 4. (FREEMAN, B.M. eds.) 165-174. Academic Press. London. 1983
- 2) FOLCH, J., M. LEES and G.H.S. STANLEY, A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., **226**: 497-509. 1957.
- 3) GRIFFIN, H.D., M.M. PERRY and A.B. GILBERT, Yolk formation. in Physiology and Biochemistry of the Domestic Fowl vol 5. (FREEMAN, B.M. eds.) 345-380. Academic Press. London. 1984.
- 4) LEVEILLE, G.A., E.K. O'HEA and K. CHAKRABARTY, *In vivo* lipogenesis in the domestic chicken. Proc. Soc. Exp. Biol. Med., **128**: 398-401. 1968.
- 5) O'HEA, E.K. and G.A. LEVEILLE, Lipid biosynthesis and transport in the domestic chick (*Gallus domesticus*). Comp. Biochem. Physiol., **30**: 149-159. 1969.
- 6) PANDA, B. and R.P. SINGH, Developments in processing quail meat and eggs. World's Poult. Sci. J., **46**: 219-234. 1990.
- 7) SNEDECOR, G.W. and W.G. COCHRAN, Statistical Methods. 7th ed. Iowa State Univ. Press. Iowa. 1980.
- 8) WATERLOW, L.C., P.J. GARLICK and D.J. MILLWARD, Protein turnover in mammalian tissues and in the whole body. 179-223. North-Holland. Amsterdam. 1978.
- 9) TANAKA, K., K. KITAHARA and T. SHIGENO, Effect of dietary protein level on lipid metabolism in growing chicks. Jpn. J. Zootech. Sci., **50**: 44-54. 1979.
- 10) TANAKA, K., N. TAKAGI, S. OHTANI and K. SHIGENO, Effect of energy increase of the diets with the addition of various levels of fat or protein on hepatic lipogenesis in growing chicks. Jpn. J. Zootech. Sci., **53**: 73-79. 1982.
- 11) HIKAMI, Y., T. SUGITA, S. HASEGAWA and T. MIZUNO, The relationship between hepatic lipogenesis and the hepatic concentration of some metabolites in chicks fed the diets containing various levels of protein and fat. Jpn. J. Zootech. Sci., **51**: 664-672. 1980.
- 12) MIZUNO, T., K. SATOH, M. SADA, Y. HIKAMI and S. HASEGAWA, Effects of the dietary protein level on *in vivo* fatty acid synthesis from glucose and amino acids and on the activities of glycolytic and gluconeogenic enzymes in liver of chicks. Jpn. J. Zootech. Sci., **53**: 124-131. 1982.
- 13) WEISS, J.F., E.C. NABER and R.M. JOHNSON, Effect of dietary fat and cholesterol on the *in vitro* incorporation of acetate-¹⁴C into hen liver and ovarian lipids. J. Nutr., **93**: 142-152. 1967.

産卵中の日本ウズラ (*Coturnix coturnix japonica*) に
おける肝臓および卵胞の脂肪酸への
[1- 14 C] 酢酸ナトリウムの取込み

喜多一美・村井篤嗣・古瀬充宏・朝倉幸孜・奥村純市

名古屋大学農学部, 名古屋市千種区 464-01

産卵中の日本ウズラ (*Coturnix coturnix japonica*) における肝臓および卵胞の脂肪酸への [1- 14 C] 酢酸ナトリウムの取込みを調査した。翼下静脈より [1- 14 C] 酢酸ナトリウムを注射し、トレーサー投与後0.5, 1, 2, 4 および6時間後に肝臓および卵胞を摘出し脂肪酸に取込まれた 14 C の放射能を測定した。肝臓の脂肪酸への [1- 14 C] 酢酸ナトリウムの取込みは、トレーサー投与後0.5時間で最も高い値を示し、投与後0.5から1時間の間で 14 C の放射能は急激に減少した。卵胞における脂肪酸の 14 C の放射能はトレーサー投与後1時間まではほとんど認められず、その後急激に高くなった。本実験より、ウズラの卵胞では脂肪酸の合成はほとんど起らず、蓄積された脂肪酸は卵胞以外において合成された脂肪酸が移行したものであると推測された。

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