«Research Note»

Time Course Changes in ^{13}C Enrichment of Respiratory CO_2 in Chickens Intravenously Single-Injected with $[1-^{13}\text{C}]$ Sodium Acetate

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Time course changes in ^{13}C enrichment of respiratory CO_2 in young chickens intravenously injected with $[1-^{13}\text{C}]$ sodium acetate were examined to clarify the rapidity of oxidation of intravenous acetate to CO_2 in chicken body. The ^{13}C enrichment reached the peak level within 20–30 minutes after single intravenous injection with 1 ml of 1% $[1-^{13}\text{C}]$ sodium acetate and returned to the initial level by 180 minutes. On the other hand, no change in ^{13}C enrichment of respiratory CO_2 was observed in control chicks injected with saline throughout the experimental period. The recovery of the injected $[1-^{13}\text{C}]$ sodium acetate was estimated to be 47.0%.

These results indicate that intravenously injected acetate into young chickens is rapidly metabolized to CO_2 and suggest a rapid use of cecal acetate as an energy source in chickens.

Key words : oxidation, intravenous acetate, ^{13}C enrichment, chicks

Introduction

Chicken and other gallinaceous birds have large ceca relative to their body size (Leopold, 1953). The ceca are a major site for bacterial fermentation activity in chickens (Annison *et al.*, 1968). Volatile fatty acids (VFAs) are produced in the ceca by microbial activity and are rapidly absorbed into the portal vein by passive diffusion (Annison *et al.*, 1968 ; Sudo and Duke, 1980). A preliminary indirect calorimetry study successfully demonstrated that $[1-^{13}\text{C}]$ sodium acetate intracecally administered into 10-week-old chickens was rapidly metabolized to CO_2 , suggesting to be utilized as an energy source in the body (Kumao *et al.*, 1999). This means that the introduced acetate is metabolized to CO_2 by microbes in the ceca and/or the intravenous acetate absorbed from the cecal wall is oxidized in the body. When $[^{14}\text{C}]$ acetate was continuously infused into the wing vein, about 6% and 11% of the total energy expenditure were probably derived from the acetate in fasted and fed adult cockerels,

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respectively (Annison *et al.*, 1969). Therefore, the latter pathway of acetate oxidation is more possible, but it is less known about the possible oxidation of intravenously administered VFAs to CO₂ in the younger chicken.

In order to clarify the rapidity of oxidation of intravenous acetate in younger chickens, time course changes of ¹³C enrichment in respiratory CO₂ was examined in chickens intravenously injected with [1-¹³C] sodium acetate.

Materials and methods

Chicks and diet : Day old male Isa Brown chicks purchased from Komatsu Breeding Farm (Matsumoto, Japan) were raised in battery brooder. They were fed a commercial formula diet (CP ≥ 21%, ME ≥ 2950 kcal/kg, Kanto Kumiai Kasei Ltd, Gunma, Japan) *ad libitum*. Water was always available. On day 21, the chicks were housed in individual cages in air-conditioned room controlled at 25°C. Feeding and watering were the same as ever.

Procedure of catheterization : Catheter (22G, Terumo Co., Tokyo, Japan) was inserted into the wing vein of 29~35-day-old chicks and elongated with polyethylene tube (Hibiki No 4, Kunii Ltd., Tokyo, Japan) of about 30 cm in length. About 1 ml of heparin (sodium heparin, 6 mg/ml) was promptly flushed through this tube, and the chick was then moved into a respiratory chamber. About 10 cm of cannula was used to exteriorize the tube, and then the tube was connected with a syringe for [1-¹³C] sodium acetate injection.

Only one measurement of ¹³C enrichment of respiratory CO₂ could be done in a single day because it takes some time to catheterize the wing vein for [1-¹³C] sodium acetate injection and 4 hours to measure ¹³C enrichment of respiratory CO₂. Immediately after being taken out from the cage (at about 9.00–10.00 am), the chick was weighed and then had no access to feed and water. Three chicks per treatment were used in this experiment with average body weight of 446.5 ± 16.8 g.

Measurement of respiratory ¹³CO₂ : The measurement was done according to the procedures described by Koh *et al.* (2001) with some modifications regarding the size of chamber and rates of gas flow. Briefly, after a catheterized chick was moved into a respiratory chamber (length 32 cm, width 29 cm and height 33 cm ; capacity 30.6 l), the chamber was then closed. A clean gas of 79% N and 21% O₂ (Okaya Sanso Co, Okaya, Japan) was flown into the chamber, while the outlet gas from the chamber was connected to diaphragm pump. The pump was equipped with 2 mass flow controllers, one of them was set to 1.09 l/min to evacuate the gas from the chamber, while another (100 ml/min) was used for sampling of gas to be directly analyzed with ¹³CO₂ analyzer (EX130S, Jasco, Tokyo, Japan). The presence of chick in the chamber gradually increased ¹²CO₂ and ¹³CO₂ concentrations, which can be monitored through ¹²CO₂ and ¹³CO₂ counts, and the concentrations became stable when total CO₂ concentration inside the chamber approaches about 1.0%. This stable condition was usually attained in 1.5 ~ 3 hours. After measurement of natural abundance (background) was done, either 1 ml of 1.0% [1-¹³C] sodium acetate (99 atom%) (Masstrace, Inc. Woburn, MA, USA) dissolved in saline or 1 ml of saline was intravenously injected in 30 seconds from a

syringe. The remaining sodium acetate in the tube was flushed out into the wing vein with about 0.4 ml heparin solution to make the acetate injection complete. CO₂ concentrations and its ¹³C enrichment were measured every 10 minutes and monitored with an infrared spectrometric method for 4 hours. ¹³C enrichment was calculated by subtracting the ¹³C atom% observed from the natural abundance value.

Calculation of recovery rate and acetate utilized : CO₂ concentration (%) of the air in the respiratory chamber and ¹³C enrichment (atom % excess) of the CO₂ were measured at 10-min-intervals from 0 to 4 hours after ¹³C acetate injection and recovery rates of each 10-min-period were summated. Recovery rate of the each 10-min-period was calculated as follows :

Recovery rate of each 10-min-period (%) = (CO₂ concentration of the 10-min-air) × (¹³C enrichment of CO₂ in the 10-min-air) × 1.19 × 10 × 1000 / 22.4 × 13 / 1.551 × 100

where 1.19 = flow rate of the air in respiratory chamber (l/min), 10 = 10 min, 1000 = conversion coefficient of g into mg, 22.4 = volume of one mole gas (l), 13 = atomic weight of ¹³C (gr), 1.551 = ¹³C injected (mg) and 100 = conversion coefficient to %.

Acetate utilized (μmol/4 hours) = (recovery rate) × 10 × 1000 / 83.03

where 10 = injected sodium acetate (mg), 1000 = conversion coefficient to μg and 83.03 = molecular weight of [1-¹³C] sodium acetate (99 atom%).

Statistical analyses : The data were subjected to analysis of variance and significance of differences between means was assayed by student *t*-test (Yanai, 1998).

Results and Discussion

The concentrations of CO₂ arising from both a clean gas flown into the chamber and CO₂ produced by the chick injected with either [1-¹³C] sodium acetate or saline in this experiment ranged from 0.75–1.05% (data are not shown). Maintaining CO₂ concentration inside the chamber below 1% is important, because higher level of CO₂ might be poisonous to the chicks.

Figure 1 shows time course changes in ¹³C enrichment of respiratory CO₂ in young chicks after single intravenous injection of [1-¹³C] sodium acetate. The ¹³C enrichment of respiratory CO₂ sharply increased and reached the peak within 20–30 minutes, gradually decreased and returned to zero-time level at about 180 minutes. On the other hand, ¹³C enrichment of respiratory CO₂ in control chickens injected with saline was unchanged throughout the experimental period. Significant differences in ¹³C enrichment of respiratory CO₂ between chicks injected with saline and [1-¹³C] sodium acetate were observed during the period of 10–90 minutes (*P* < 0.05). This means that a considerable amount of the injected acetate is metabolized to ¹³CO₂ during the first 90 minutes. According to our previous study (Kumao *et al.*, 1999) using 10-week-old chicks intracecally introduced with [¹³C] acetate, a peak level of ¹³C enrichment of respiratory CO₂ appeared in 80 minutes. The relatively rapid appearance of the peak ¹³C enrichment in the present study is possibly due to the difference in age and route of acetate administration. The chick has a higher rate of tissue metabolism during the early age, including oxidation and synthesis of substrates into cellular components to meet the requirements for rapid growth. This is supported by the *in vitro* study that

Fig. 1. Time course changes of ^{13}C enrichment of intravenous $[1-^{13}\text{C}]$ sodium acetate or saline in chicks. Results are means $\pm$ SD for 3 chicks for each experimental point. There is a significant difference between a and b at each time ($P < 0.05$).

palmitate oxidation in the adipose tissue as well as glucose oxidation in the liver decreases with an increase in age from 4 to 8 or 12-week-old broilers (Vasilatos-Younken, 1986). Another possibility is that different routes were employed for the administration of $[1-^{13}\text{C}]$ sodium acetate, namely intravenous and intracecal routes. The delay in peak of ^{13}C enrichment for the intracecal administration of acetate should be attributed to the time taken for absorption into the portal blood.

Table 1 shows the rate and the amount of ^{13}C recovered in the respiratory CO_2 in chickens injected with $[1-^{13}\text{C}]$ sodium acetate. The recovery rate and the amount were 47.04% and 56.65 μM , respectively. The recovery level of the injected acetate was similar to 43% in the previous study (Kumao *et al.*, 1999). Similar rates of acetate utilization in both studies suggest that intracecal acetate is rapidly absorbed into the portal blood and metabolized to CO_2 in chicken body. According to Legrand *et al.* (1987) radioactivity of ^{14}C was largely found in the liver and plasma and also distributed in the muscle and adipose tissue even 8 hours after intravenous administration of ^{14}C -acetate to genetically lean or fat chickens. Therefore, the remaining of the injected ^{13}C in the present study may accumulate in these tissues as metabolites.

These results indicate that intravenous acetate is rapidly metabolized to CO_2 , and suggest the rapid use of cecal acetate as an energy source in chickens.

Table 1. The rate and the amount of ^{13}C recovered as CO_2 in 4-week-old chicks fed a commercial diet intravenously injected with $[1-^{13}\text{C}]$ sodium acetate

	Recovery rate	Acetate utilization
	%	$\mu\text{M}/4$ hours
Mean $\pm$ SD*	47.07 $\pm$ 2.59	56.92 $\pm$ 3.13
Range	44.16 – 49.16	53.43 – 59.49

* Data from 3 chicks

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