Comparative Effects of Intravenously Single-injected D-[U-\(^{13}\)C]Glucose and [1-\(^{13}\)C]Sodium Acetate on Time Course Changes in \(^{13}\)C Enrichment of Respiratory CO\(_2\) in Growing Chickens

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This study was carried out to examine which of intravenous glucose or acetate would be rapidly oxidized to CO\(_2\) in growing chickens fed a semi-purified diet.

\(^{13}\)C Enrichment of respiratory CO\(_2\) in chickens intravenously single-injected with [1-\(^{13}\)C]sodium acetate sharply increased and reached the peak level within 30–40 min, then almost returned to the initial level by 170 min. On the other hand, the peak level in chickens intravenously injected with D-[U-\(^{13}\)C]glucose was observed at about 100 min and did not return to the initial level even at 4 h. The peak level of \(^{13}\)C enrichment in chickens injected with [1-\(^{13}\)C]sodium acetate was about 2.3 times higher than that in chickens injected with D-[U-\(^{13}\)C]glucose. The rate of \(^{13}\)C recovered in the respiratory CO\(_2\) for 4 h tended to be higher in chickens injected with [1-\(^{13}\)C]sodium acetate than in those injected with D-[U-\(^{13}\)C]glucose, although the difference was not significant.

Rapid appearance of \(^{13}\)C in the respiratory CO\(_2\) from [1-\(^{13}\)C]sodium acetate more than D-[U-\(^{13}\)C]glucose suggests that cecal acetate is utilized as effective as dietary glucose in the chicken body.

**Key words**: growing chicken, glucose oxidation, acetate oxidation, respiratory \(^{13}\)CO\(_2\)

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**Introduction**

Dietary carbohydrate is mainly comprised of starch and a small amount of non-starch polysaccharides (NSP). The dietary indigestible NSP, particularly soluble type, is fermented to produce volatile fatty acids (VFAs) by microorganisms in the hindgut, mainly in the ceca where the most bacterial counts are observed in the chicken intestine (Mead, 1997). Acetate of a main cecal VFA is absorbed into the portal blood (Annison et al., 1968) and possibly oxidized and utilized as energy source in chicken
body. Legrand et al. (1987) reported that about 60% of the injected [1\(^{14}\)C]acetate was recovered in the respiratory CO\(_2\) in 9-week-old chickens for the period of 8 h after injection. We have also found that 47% of intravenously single-injected [\(^{1-13}\)C]acetate was recovered in the respiratory CO\(_2\) in 4-week-old chickens (Widodo et al., 2003).

Annison et al. (1969) reported that glucose could contribute to a much larger extent to overall energy expenditure than acetate when glucose and acetate were continuously infused into mature chickens fed a commercial diet, whereas the contribution of glucose and acetate were similar in fasted chickens. Swart et al. (1993) observed that orally dosed [\(^{14}\)C]acetate was more rapidly oxidized than [\(^{14}\)C]glucose in ostriches fed a commercial diet, and suggested that the difference might be due to different absorption rates from the intestine. We have also found that 47.8% of intravenously single-injected D-[\(^{13}\)C]glucose was recovered in the form of CO\(_2\) in 3-month-old chickens for 5 h after the injection (Koh et al., 2001). However, comparison of rapidity of acetate and glucose oxidation under the same feeding conditions has not been documented in the chicken.

In order to elucidate the role of intra-cecal VFAs as energy source, the oxidation of intravenous D-[\(^{13}\)C]glucose and [\(^{1-13}\)C]sodium acetate were compared in growing chickens.

**Materials and Methods**

*Animals and diets*

Day-old male ISA Brown chicks were commercially obtained (Komatsu Breeding Farm, Matsumoto, Japan) and raised in battery brooder. They were given a commercial formula diet (CP\(\geq 21\%\), ME\(\geq 2950\) kcal/kg, Kanto Kumiai Kasei Ltd, Gunma, Japan) and water *ad libitum*. At 21 d of age, the chickens were housed in individual cages in air-conditioned room controlled at 25\(^\circ\)C. Four, five and two chickens ranging from 27 to 33 d of age, with average body weight of 358 g, were allotted to acetate, glucose and saline-injection groups, respectively. All of the experimental birds were fed the same semi-purified diet for 7 d prior to the injection, except for two chickens in the acetate-injected group fed the diet for 8 and 9 d. The semi-purified diet contained 20% crude protein and 3264 kcal ME/kg, and its composition was as follows (g/kg): isolated soy-protein (Fuji Seiyu Ltd, Osaka, Japan) 243.1, cornstarch (Nihon Cornstarch Ltd, Aichi, Japan) 634.19, cellulose (Koujin Ltd, Toyama, Japan) 35, corn oil 20, cystine 0.9, methionine 0.46, NaHCO\(_3\) 10, choline chloride 2, dl-\(\alpha\)-tocopherol acetate 0.02, 2,6-di-t-butyl-p-cresol 0.13, vitamin mixture (Velu et al., 1971) 0.6 and mineral mixture (Velu et al., 1971) 53.6. The experimental birds had free access to water and diet all the time. We treated all experimental animals according to the guideline for regulation of animal experimentation of Shinshu University, Japan.

*Catheter*

Catheter (22G, Terumo Co., Tokyo, Japan) was inserted into the wing vein 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 saline) was flushed into the tube immediately after the procedure, and the chicken was put into a respiratory
About 10 cm of cannula was used to exteriorize the tube and connected with a syringe for injection with [1,13C]sodium acetate, D-[U-13C]glucose or saline solution. Only one measurement of 13C enrichment in respiratory CO2 was done in a single day, because it took some time for catheterization and 4 h to measure 13C enrichment of respiratory CO2.

**Measurement of respiratory 13CO2**

The measurement of respiratory 13CO2 was done according to the procedure described by Widodo et al. (2003). In the previous study CO2 concentration slightly exceeded 1.0% in the respiratory chamber and so inlet gas flow rate was adjusted to keep CO2 concentration below 1.0%. In addition, the chicken stayed overnight in the chamber for acclimatization before measurement. These improvements made it possible to shorten the time taken for measurement of natural abundance, about 1-2 h in this study vs. 1.5-3 h in the previous study.

After measurement of natural abundance (background), the chicken was intravenously single-injected with any of 1 ml of 120 μmol [1,13C]sodium acetate (99 atom %) (Masstrace, Inc. Woburn, MA, USA), 1 ml of 55 μmol D-[U-13C]glucose (98 atom %) (Shoukou Tsushou Ltd, Tokyo, Japan) and 1 ml of saline/350 g body weight in 30 sec. Remaining solution in the tube was flushed out into the wing vein with about 0.4 ml heparin solution to make injection complete. 12CO2 and 13CO2 were measured every 10 min and monitored with an infrared spectrometric 13CO2 analyzer (Jasco EX-130S, Jasco Corp, Tokyo, Japan) for 4 h. 13C Enrichment was obtained by subtracting the natural abundance value from the 13C atom% determined. Other calculations for the rate and the amount of the 13C recovered as CO2 were done according to Widodo et al. (2003).

**Statistical analysis**

13C Enrichment data were subjected to one-way ANOVA and further significance test of differences among mean values by using Fisher’s Protected Least Significant Difference (PLSD) test (Yanai, 1998). While means of the 13C recovered as respiratory CO2 in chickens injected with D-[U-13C]glucose and [1-13C]sodium acetate were analyzed by using student t test (Yanai, 1998).

**Results**

The concentration of CO2 arising from CO2 produced by the chicken injected with [1,13C]sodium acetate, D-[U-13C]glucose or saline ranged from 0.80% to 1.03% in this study (data are not shown). The CO2 concentration inside the chamber suggests that possible adverse effect of CO2 on the chicken should be avoided in the present study.

Figure 1 shows time course changes of 13C enrichment in respiratory CO2 in chickens after intravenous single injection with [1-13C]sodium acetate, D-[U-13C]glucose and saline. This study demonstrated that the intravenous injection with [1-13C] sodium acetate and D-[U-13C]glucose caused different time course patterns of 13C enrichment in respiratory CO2 in chickens. The 13C enrichment of respiratory CO2 in chickens injected with [1-13C]sodium acetate sharply increased and reached the peak level within 30-40 min, then gradually decreased and returned to almost zero-time level.
after about 170 min. On the other hand, in chickens injected with D-[U-\(^{13}\)C]glucose the \(^{13}\)C enrichment of respiratory CO\(_2\) slightly increased and reached the peak level at about 100 min, then decreased but did not return to zero-time level at 4 h. In addition, the \(^{13}\)C enrichment of respiratory CO\(_2\) in the chickens injected with [\(^{1-13}\)C]sodium acetate was significantly higher (P < 0.05) than that injected with D-[U-\(^{13}\)C]glucose during the period of 10–80 min, but \(^{13}\)C enrichment in [\(^{1-13}\)C]sodium acetate group was significantly lower (P < 0.05) than that in D-[U-\(^{13}\)C]glucose group during 110–240 min. The peak level of \(^{13}\)C enrichment in chickens injected with [\(^{1-13}\)C]sodium acetate was about 2.3 times higher than that in chickens injected with D-[U-\(^{13}\)C]glucose. In control chickens injected with saline, \(^{13}\)C enrichment of respiratory CO\(_2\) remained unchanged throughout the experimental period.

Table 1 shows the recovery rate of \(^{13}\)C in the respiratory CO\(_2\) of growing chickens for 4 h after intravenous injection with D-[U-\(^{13}\)C]glucose or [\(^{1-13}\)C]sodium acetate. Average recovery rates of \(^{13}\)C for 4 h were 45.5% and 66.4%, in chickens injected with D-[U-\(^{13}\)C]glucose and [\(^{1-13}\)C]sodium acetate respectively, but the difference was not significant.

**Discussion**

Microbial fermentation of non-starch polysaccharides in the ceca of domestic fowls has been reported to produce volatile fatty acids (VFA). Acetate of a main cecal VFA is absorbed into the portal blood (Annison *et al.*, 1968) and possibly oxidized and
utilized as energy source in chicken body. Annison et al. (1969) also reported that glucose could contribute to a much larger extent to overall energy expenditure than acetate when it was continuously infused into mature chickens fed a commercial diet. Studies with D-[U-13C]glucose (Koh et al., 2001) and [1-13C]acetate (Widodo et al., 2003) indicated a possible use of the intravenously injected acetate and glucose as energy source in growing chickens. However, the rapidity of acetate and glucose oxidation under the same feeding conditions has not been compared in the growing chickens. In the present study, the attainment of the peak level of 13C enrichment in the respiratory CO2 under the same feeding condition was 60 min earlier in chickens injected with [1-13C]acetate than in those injected with D-[U-13C]glucose. The results suggest that intestinal acetate is more rapidly oxidized to CO2 than intestinal glucose in chicken body once they are absorbed from the intestinal wall.

Comparative study on the metabolic rates of acetate, lactate and glucose suggested that acetate and lactate were oxidized to CO2 at greater rates than glucose was in both liver and adipose tissue slices taken from praire voles, an herbivorous rodent (Baldner et al., 1984). Moreover, [1-14C]acetate (Leville, 1967) as well as [1-13C]acetate (Wolfe and Jahoor, 1990) is more likely to be oxidized through TCA cycle, rather than to be utilized for fatty acid and cholesterol synthesis. The greater oxidation rate to CO2 and the preferential oxidative pathway through TCA cycle may account for the finding that intravenous acetate is more rapidly oxidized to CO2 than glucose.

The injected amount of 13C in glucose was about 3 times larger than that in acetate (4.27 mg/ml vs 1.56 mg/ml), because all glucose carbons were universally labeled and an injected molar amount of glucose was half the amount of acetate. However, the peak level of 13C enrichment of respiratory CO2 in chickens injected with [1-13C]acetate was about 2.3 times higher than that in chickens injected with D-[U-13C]glucose. The low peak in 13C enrichment in the 13C glucose injection might be attributable to a large glucose pool in the chicken body. The comparative study on lipid biosynthesis from acetate and glucose in chickens suggests that acetate pool is smaller than glucose pool (O’Hea and Leville, 1969). Another evidence is that plasma glucose concentration in chickens is extraordinarily high (250 mg/dl for 8-week-old chickens, Riesenfield et al., 1982) as compared with those of rats (65 mg/dl, Frias and Sgarbieri, 1998) and humans (80–100 mg/dl, Mayes, 1985). The large glucose pool is also unchangeable as explained by that blood glucose concentration remains unchanged even in the chickens fasted (Belo et al., 1976) and fed high carbohydrate (Renner, 1964) and carbohydrate-free

<table>
<thead>
<tr>
<th>Injected</th>
<th>Number of chickens</th>
<th>Recovery rate1 %</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>5</td>
<td>45.5± 8.22</td>
</tr>
<tr>
<td>Acetate</td>
<td>4</td>
<td>66.4±14.0</td>
</tr>
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1[13C recovered in the respiratory CO2]/[13C injected]. 2Values are means±SD.
diets (Allred and Roehrig, 1970). These data suggest the largeness and steadiness of glucose pool of the chicken. Therefore, the large glucose pool and its homeostasis mechanism are possibly involved in the less changeable time course pattern of $^{13}$C enrichment in the respiratory CO$_2$ in chickens injected with D-$[U-^{13}$C]glucose.

The rate of $^{13}$C recovered in the respiratory CO$_2$ in chickens injected with [1-$^{13}$C] sodium acetate in the present study was 66.4%, which is similar to that reported by Legrand et al. (1978). However, this rate is higher than that in our previous report (Widodo et al., 2003) (66.4 vs 47.0%). The higher recovery rate of $^{13}$C in the respiratory CO$_2$ in the present study may be particularly attributed to different diets used, because other experimental conditions were similar. The semi-purified diet used in this study increases cecal VFAs concentrations (Shiba et al., unpublished data). The commercial diet used may cause increases in endogenous substances like exfoliated epithelial cell, pancreatic enzymes, mucins and other intestinal secretion and dead bacterial cell. The presence of these substances would make more difficult for microflora to ferment dietary substrates such as non-starch polysaccharides to produce VFA. Presumably, the increased amount of VFAs absorbed from the cecal wall would stimulate the uptake of blood VFAs by the liver, and could enhance VFAs oxidation to CO$_2$. This is also supported by the perfused rat hind-leg study that acetate uptake is linearly correlated with initial concentrations of acetate in the perfusion medium, and the increased acetate uptake accordingly stimulates oxidation of [U-$^{14}$C]acetate to CO$_2$ (Karlsson et al., 1975).

When D-$[U-^{13}$C]glucose was intravenously injected, the rate of $^{13}$C recovered in the respiratory CO$_2$ was similar in 4-week-old chickens fed a semi-purified diet and in 3-month-old cockerels fed a commercial diet (Koh et al., 2001). Taking into consideration on large glucose pool and glucose homeostasis in the chicken, this result suggests that glucose oxidation in the chicken body is not affected by age and diet.

Rapid appearance of $^{13}$C in the respiratory CO$_2$ from [1-$^{13}$C]acetate more than D-$[U-^{13}$C]glucose suggests that acetate is as effective energy source as glucose in the growing chicken.

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References

Baldner GL, Beitz DC and Hood RL. Conversion of glucose, acetate and lactate to CO$_2$ and fatty


