Effect of Dietary Isoleucine Level and Food Intake on Energy Utilization by Growing Male Chicks

Kunio Sugahara and Tatsuo Kubo

Department of Animal Science, Faculty of Agriculture, Utsunomiya University, Utsunomiya-shi 321

Two experiments involving comparative slaughter procedures were conducted to see if the decrease in total energy retention (ER) resulted from the decreased food intake in growing chicks fed on a diet containing isoleucine less than its requirement. Ad libitum feeding a diet containing 3.0 g of isoleucine/kg (50% deficiency) decreased food intake, body weight gain, apparent metabolizable energy (AME) intake, ER, and ratio of ER/AME intake. When both the control diet and the diet containing 3.0 g isoleucine/kg were tube-fed at the high and low levels of food intake (218.5 and 74.8 g/chick/14 days, respectively), body weight gain significantly lower in chicks on the low-isoleucine diet than in the control chicks at each level of intake. Energy retained as protein was significantly decreased by the low-isoleucine diet and reducing food intake. Energy retained as fat was affected by food intake but not by dietary isoleucine level. Energy retention was unaffected by dietary isoleucine level and depended on AME intake alone. No main effect of dietary isoleucine level on the ratio of ER/AME intake or heat increment by feeding was observed but significant interactions between isoleucine and food intake effects were detected in both the criteria. These results indicate that the decreased ER in chicks fed on the low-isoleucine diet was due mainly to the decreased food intake and not to the decreased efficiency of AME utilization.


Key words: isoleucine deficiency, food intake, energy retention, chick

Introduction

Deficiencies of some single essential amino acids decrease food intake, body weight gain, and total energy retention (ER). Tasaki et al. (1972) and Yanaka and Tasaki (1980) showed the efficiency of apparent metabolizable energy (AME) utilization to be a main factor affecting ER in growing chicks fed on a diet deficient in lysine or sulfur-containing amino acids (SAA). We have demonstrated, by tube-feeding diets containing two concentrations of some amino acids at two rates of food intake, that the decreased ER in chicks fed on a diet deficient in arginine, lysine, SAA, tryptophan or threonine was associated with decreased food intake (Sugahara et al., 1985; Sugahara and Kubo, 1992a, b; Kubo and Sugahara, 1995). Dietary requirement of isoleucine for growing broilers has been reported by Farran and Thomas (1990) and Barbour and Latshaw (1992) based on weight gain and feed conversion. Velu et al. (1972) reported that isoleucine deficiency increased the carcass fat content in equally-fed chicks. However, little is known concerning the effect of dietary isoleucine level on energy utilization by growing chicks. In order to determine if levels of dietary isoleucine...
affect the efficiency of energy utilization by growing chicks, an *ad libitum* feeding trial with four levels of isoleucine was conducted (Experiment 1). OKUMURA and MORI (1985) have reported that energy retention by chicks receiving the isoleucine-deficient diet increased as food consumption increased without controlling the feeding pattern. We examined their results under the condition that the amount and pattern of food intake were controlled with the tube-feeding method (Experiment 2).

**Materials and Methods**

Two experiments were conducted; the procedures were identical in the two experiments unless otherwise indicated. In each experiment 200 1-d-old male layer-strain chicks were fed *ad libitum* on a commercial chick starter diet (CP 200 g/kg, AME 12.1 MJ/kg) for 4 days. At 5 days of age 48 chicks were selected according to body weight after 3 hours of starvation. They were fed *ad libitum* (Experiment 1) or tube-fed 3 times a day (Experiment 2) on a control diet for the following three days. At 8 days of age, 25 chicks were selected from the 48 chicks and were divided into five groups of 5 chicks so that the initial body weight was as uniform as possible. The four groups were individually housed in metabolism cages and randomly allocated to experimental treatments. Experimental diets were fed from 8 to 22 days of age. The other group was killed by ether inhalation for analysis of initial body composition. The treatments in Experiment 1 included the four dietary isoleucine levels (6.0, 4.5, 3.0 and 1.5 g/kg) with *ad libitum* feeding. In Experiment 2, a 2×2 factorial experimental design (dietary isoleucine level: 3.0 and 6.0 g/kg and food intake level: 74 and 218.5 g/chick/14 days) was used. The high and low levels of food intake were designed to be equal to the voluntary food consumption of chicks fed on the diets containing 6.0 and 3.0 g isoleucine/kg, respectively, in Experiment 1. The method of tube-feeding was used to control the amount and pattern of food intake. The diets were mixed with water in the ratio of 1:0.66 (wt/wt) immediately before they were administered into the crop using a syringe with a tube three times daily, at 9:00, 13:00, and 18:00 hours.

The composition of the control diet was similar to that described in our previous report (KUBO and SUGIHARA, 1995). The diets containing 1.5, 3.0, and 4.5 g isoleucine/kg were formulated by reducing 75, 50, and 25% of isoleucine of the control diet (6.0 g/kg), respectively. The CP and gross energy (GE) levels of the experimental diets were kept at 145 g/kg and 18.5 MJ/kg, respectively.

Energy retained as protein (ERP) and as fat (ERF) were derived by calculation from protein (nitrogen×6.25) and fat retention with energy coefficients of 23.68 kJ/g for protein and 39.12 kJ/g for fat. ER was a sum of ERP and ERF. The procedures for grinding carcasses and determining their nitrogen and fat contents were similar to those of our previous study (SUGAHARA *et al.*, 1984). Heat increment was calculated as the difference between ER plus starving heat production and AME intake. Starving heat production was estimated to be 400 kJ/kg^{0.75}/day (SUGAHARA *et al.*, 1984; SUGAHARA *et al.*, 1988) and assumed not to be affected by dietary isoleucine level. Dietary AME value was calculated as the difference of the GE of the diets eaten and the excreta voided during the last 4 days of the experiments.
Lighting was provided continuously with fluorescent lamps. The environmental temperature ranged from 32 to 35°C. Data were analyzed by analysis of variance and treatment differences were evaluated by Tukey’s test (SAS Institute, 1985).

Results and Discussion

The effects of the dietary isoleucine level on food intake, body weight gain, feed efficiency (gain/feed), and energy utilization by growing chicks are presented in Table 1. Food intake and body weight gain were increased by increasing isoleucine up to 6.0 g/kg diet. AME, ERP, ERF, and ER increased with an increment in dietary isoleucine level. The ratio of AME/GE was unaffected by the dietary isoleucine concentrations. Feed efficiency at the levels of 6.0 and 4.5 g isoleucine/kg were similar and larger than that at the level of 3.0 g isoleucine/kg. Response in the ratio of ER/AME intake to the dietary isoleucine level was similar to that in feed efficiency. Chicks fed on the diet containing 1.5 g isoleucine/kg could not maintain their initial body weight. In general, the effects of ad libitum feeding the diet containing graded levels of isoleucine on the food intake, body weight gain, feed efficiency and efficiency of energy utilization are in agreement with the results of VELU et al. (1972) and those of SUGAHARA and KUBO (1992 b) for tryptophan, but different from those for arginine (SUGAHARA et al. 1985).

The criteria studied here, except feed efficiency and the ratios of AME/GE and ER/AME intake, were significantly lower at 4.5 than 6.0 g isoleucine/kg. The drastic differences in those criteria and significant difference in the ratio of ER/AME intake between the levels of 4.5 and 3.0 g isoleucine/kg were observed, indicating that level of 3.0 g isoleucine/kg was considered to be critical for growing chicks in terms of energy utilization. Similar responses have been observed in chicks on the diets deficient in arginine, lysine, SAA, tryptophan or threonine (SUGAHARA et al., 1985 ; SUGAHARA and

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### Table 1. Effect of feeding ad libitum diets containing graded levels of isoleucine on body weight gain, food intake, gain/feed, and energy utilization (Experiment 1)

<table>
<thead>
<tr>
<th>Dietary isoleucine level</th>
<th>Food intake</th>
<th>Body weight gain</th>
<th>Gain/feed</th>
<th>AME/GE intake</th>
<th>ERP</th>
<th>ERF</th>
<th>ER</th>
<th>ER/AME intake</th>
<th>Heat increment</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/kg)</td>
<td>(g/chick/14 days)</td>
<td>(g/g)</td>
<td>(kJ/kj)</td>
<td>......</td>
<td>(kJ/chick/14 days) ......</td>
<td>(kJ/kj)</td>
<td>(kJ/100kJ AME intake)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>210.9a</td>
<td>112.0a</td>
<td>0.53a</td>
<td>0.91a</td>
<td>3547a</td>
<td>459a</td>
<td>930a</td>
<td>1389a</td>
<td>0.39a</td>
</tr>
<tr>
<td>4.5</td>
<td>139.7b</td>
<td>51.9b</td>
<td>0.37a</td>
<td>0.90a</td>
<td>2294b</td>
<td>231b</td>
<td>376b</td>
<td>607b</td>
<td>0.27a</td>
</tr>
<tr>
<td>3.0</td>
<td>69.2c</td>
<td>3.7c</td>
<td>0.04b</td>
<td>0.91c</td>
<td>1165c</td>
<td>34c</td>
<td>5c</td>
<td>39c</td>
<td>0.03b</td>
</tr>
<tr>
<td>1.5</td>
<td>33.5d</td>
<td>-15.3d</td>
<td>-0.48c</td>
<td>0.89c</td>
<td>552d</td>
<td>-18d</td>
<td>-181d</td>
<td>-199d</td>
<td>-0.38d</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>5.5</td>
<td>3.2</td>
<td>0.04</td>
<td>0.01</td>
<td>99</td>
<td>11</td>
<td>38</td>
<td>47</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Means within columns with different superscripts differed significantly (P<0.05).
1 Mean of five chicks per treatment. Average initial body weight was 72.5 g.
2 Average initial body protein, fat, and water were 16.2%, 9.4% and 72.4%, respectively.
3 Energy retained as protein
4 Energy retained as fat
5 Energy retention = ERP + ERF
6 AME intake = (energy retention + 400 x body weight (kg)^0.75).
Kubo 1992a, b; Kubo and Sugahara, 1995). In Experiment 2 the level of 3.0 g isoleucine/kg was used to examine the effects of the dietary isoleucine level and food intake on the energy utilization.

The results of tube-feeding the diet containing 3.0 or 6.0 g isoleucine/kg are summarized in Table 2. Chicks tube-fed on the low-isoleucine diet (3.0 g isoleucine/kg) at the high level of intake were forced to be given about three times the diets which they could eat under ad libitum feeding. The degree to which food intake was decreased by ad libitum feeding the isoleucine-deficient diet was greater compared to that resulted from single deficiencies of other essential amino acids studied by the authors (Sugahara et al., 1985; Sugahara and Kubo 1992a, b). No birds regurgitated, probably because they had been trained to be tube-fed for the last three days before the experiment started.

There was a significant (P<0.01) interaction between the effects of food intake and isoleucine on body weight gain. The effect of isoleucine concentration on the body weight gain was greater at the high food intake. Body weight gain in chicks receiving the low-isoleucine diet was much larger at the high level of intake than at the low intake, but still significantly (P<0.05) smaller than the control counterparts. These findings indicate that food intake accounted for a part of the decreased body weight gain resulting from the isoleucine deficiency. This is in agreement with our previous studies on other essential amino acids (Sugahara et al., 1985; Sugahara and Kubo, 1992a, b; Kubo and Sugahara, 1995) and the study by Kino and Okumura (1986). Feed

Table 2. Effect of levels of isoleucine and food intake on body weight gain, gain/feed, and energy utilization by tube-fed chicks (Experiment 2)1

<table>
<thead>
<tr>
<th>Food intake level</th>
<th>Dietary isoleucine level (g/kg)</th>
<th>Body weight gain (g/chick/14 days)</th>
<th>Gain/feed (g/g)</th>
<th>AME intake (kJ/kg)</th>
<th>AME retention (kJ/chick/14 days)</th>
<th>ERP (kJ/kg)</th>
<th>ERF (kJ/kg)</th>
<th>ER (kJ/kg)</th>
<th>Heat increment (kJ/100kJ AME intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>6.0</td>
<td>95.5</td>
<td>0.44</td>
<td>0.86</td>
<td>3455</td>
<td>353</td>
<td>1978</td>
<td>0.57</td>
<td>9.4</td>
</tr>
<tr>
<td>High</td>
<td>3.0</td>
<td>95.5</td>
<td>0.44</td>
<td>0.86</td>
<td>3455</td>
<td>353</td>
<td>1978</td>
<td>0.57</td>
<td>9.4</td>
</tr>
<tr>
<td>Low</td>
<td>6.0</td>
<td>17.7</td>
<td>0.24</td>
<td>0.92</td>
<td>1249</td>
<td>164</td>
<td>303</td>
<td>0.24</td>
<td>9.1</td>
</tr>
<tr>
<td>Low</td>
<td>3.0</td>
<td>11.3</td>
<td>0.15</td>
<td>0.91</td>
<td>1251</td>
<td>71</td>
<td>297</td>
<td>0.23</td>
<td>10.2</td>
</tr>
<tr>
<td>Pooled SE</td>
<td>1.7</td>
<td>0.01</td>
<td>0.006</td>
<td>0.006</td>
<td>21</td>
<td>10</td>
<td>36</td>
<td>0.01</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Analysis of variance

<table>
<thead>
<tr>
<th>Food intake level</th>
<th>Isoleucine</th>
<th>Food intake</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
<tr>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 Mean of five chicks per treatment. Average initial body weight was 79.7 g.

2 Energy retained as protein
3 Energy retained as fat
4 Energy retention = ERP + ERF
5 AME intake - (energy retention + 400 × body weight (kg)0.75)
6 218.5 g/chick/14 days.
7 74.8 g/chick/14 days.
** P<0.01, * P<0.05, NS Not significant (P>0.05).
efficiency was significantly (P<0.01) lower in chicks on the low-isoleucine diet than in the control chicks at each level of food intake. Feed efficiency was greater at the high food intake level than at the low level.

A significant (P<0.01) interaction between food intake and isoleucine effects occurred in the AME/GE ratio. The ratio of AME/GE of the control diet was not affected by the level of food intake. When chicks were tube-fed the isoleucine-deficient diet at the high intake level, AME/GE ratio was reduced. This interaction has been observed in deficiencies of lysine (SUGAHARA and KUBO, 1992a) and tryptophan (SUGAHARA and KUBO, 1992b) but not of arginine (SUGAHARA et al., 1985), SAA (SUGAHARA and KUBO, 1992a), and threonine (KUBO and SUGAHARA, 1995). The excessive intake of some amino acids may have some ill effects, because AME/GE ratio was not affected when the diet was given ad libitum or at the lower level by tube-feeding. The effect of the levels of food intake and dietary isoleucine on AME intake was similar to that on AME/GE ratio.

Significant (P<0.05) main effects of isoleucine and food on ERP were observed. Irrespective of the dietary isoleucine level, ERP was higher at the high intake level than at the low level. Chicks tube-fed on the low-isoleucine diet showed lower ERP than those on the high-isoleucine diet at each intake level. Although no significant interaction between isoleucine and food intake occurred in ERP in the present experiments, this interaction has been observed with single deficiencies of arginine, lysine, SAA (SUGAHARA et al., 1985; SUGAHARA and KUBO, 1992a) and threonine (KUBO and SUGAHARA, 1995) but not with tryptophan (SUGAHARA and KUBO, 1992b). Whether or not this interaction was observed probably depends on amino acids tested.

Energy retained as fat was significantly (P<0.01) greater at the high level of intake than at the low. The isoleucine-deficient chick had more ERF, though the difference was not significant (P>0.05). The increased ERF by forced-feeding the diets deficient in some essential amino acids has been reported (SUGAHARA et al., 1985; KINO and OKUMURA, 1986; SIBBALD and WOLYNETZ, 1986; SUGAHARA and KUBO, 1992a). Carcass fat concentration was significantly (P<0.05) larger in the deficient chicks than in the control chicks (data are not shown in table). This result is in agreement with the result of VELU et al. (1972).

Energy retention was significantly (P<0.01) larger at the high intake than at the low intake, regardless of isoleucine deficiency, and depended on food intake. The present results are in agreement with those of OKUMURA and MORI (1985).

A significant (P=0.037) interaction between isoleucine and food intake effects occurred on the ratio of ER/AME intake. The degree to which the ratio of ER/AME intake increased with an increment in food intake was greater with the low isoleucine level than with the control level. When chicks were fed on the diets at the high intake level, the isoleucine-deficient group had greater ratio of ER/AME intake than the control group. There was no significant (P>0.05) difference in the ratio of ER/AME intake between the control and isoleucine-deficient chicks when they were given the diets at the low food intake level. This ratio, nevertheless, was significantly (P<0.05) greater at the high intake level than in the low intake at both the two dietary
isoleucine levels. These results suggest that ER was in proportion to AME intake irrespective of the dietary isoleucine level.

Neither dietary isoleucine level nor food intake affected heat increment of feeding. A significant (P = 0.048) interaction between isoleucine and food intake effects was observed on heat increment. Heat increment in the control group was larger at the high intake level than at the low intake, but heat increment in the isoleucine-deficient group was not different between the two levels of food intake. It is well known that heat increment increases with an increment in food intake. The reason why no significant (P > 0.05) difference in heat increment by the isoleucine-deficient group was observed between the two food intake levels is not known. Because the P value obtained was close to 0.05, and because single deficiencies of arginine, lysine, SAA, tryptophan, or threonine did not affect heat increment (SUGAHARA et al., 1985; SUGAHARA and KUBO, 1992a, b; KUBO and SUGAHARA, 1995), it is likely that isoleucine deficiency may not affect heat increment. The absence of an effect of isoleucine deficiency on heat increment indicates that the partial efficiency of AME utilization would be similar in the control and deficient chicks.

The results of the present experiments indicated that the effect of isoleucine deficiency on the energy retention was associated mainly with food intake and confirm the findings of OKUMURA and MORI (1985). The decreased energy retention by the isoleucine-deficient chicks could not be explained by the heat increment by feeding in the present experiments. The conclusion derived from the present experiments is quite similar to that of our previous reports on single deficiencies of other essential amino acids (SUGAHARA et al., 1985; SUGAHARA and KUBO, 1992a, b; KUBO and SUGAHARA, 1995).

References


