Phenylalanine Requirement, Imbalance, and Dietary Excess in One-Week-Old Chicks: Growth and Phenylalanine Hydroxylase Activity

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ABSTRACT Two experiments were performed to study Phe imbalance and toxicity in 1-wk-old Babcock B380 chicks resulting from the addition of either a mixture of indispensable amino acids lacking Phe (IAA – Phe) or excess Phe to a diet that was nutritionally adequate in Phe. Chicks received a preexperimental semipurified diet for 1 wk and experimental diets from 7 to 14 d of age. In the first experiment, the chicks were given diets with Phe levels at 0.24, 0.29, 0.34, 0.39, 0.44, and 0.49% of the diet to determine the Phe requirement. The requirement of the chicks for Phe, based on weight gain and feed efficiency, was determined to be 0.39% of the diet. In experiment 2, the IAA – Phe (10% of the diet) or excess Phe (2% of the diet) was added to a diet containing 0.44% Phe. Chicks given the IAA – Phe or excess Phe had significantly slower growth rates than chicks given the basal diet (P ≤ 0.05). The activities of the major hepatic enzyme of Phe catabolism, Phe hydroxylase (PAH), were significantly higher than that of chicks fed the basal diet when the chicks were fed the diets containing IAA – Phe plus 1.1% Phe (P ≤ 0.05) but not when chicks were fed the diet containing IAA – Phe alone. The activity of PAH in chicks given the excess (2%) Phe was nearly 4 times the activity of PAH in chicks given the basal diet. Adding IAA – Phe to the diet containing excess Phe also resulted in higher PAH activity than was observed in chicks fed the basal diet, although the activity was significantly lower than observed for chicks receiving the diet containing excess Phe alone (P ≤ 0.05). It is concluded that hepatic PAH activity in chicks increases primarily in response to its substrate, Phe. A dietary amino acid load without Phe reduces this response to excess Phe.

Key words: phenylalanine hydroxylase, imbalance, toxicity, phenylalanine requirement

INTRODUCTION

An amino acid imbalance is the growth depression that occurs when a diet that is first-limiting in an amino acid is supplemented with the second-limiting amino acid or a mixture of amino acids lacking the first limiting one (Harper, 1956). Amino acid toxicity is the growth depression that results from the ingestion of a diet containing an excess of a particular amino acid (Harper, 1956).

In virtually all cases of amino acid imbalances, in addition to the growth depression, there is a decrease in feed intake and the plasma concentration of the amino acid under investigation (Harper et al., 1970). In the cases of Thr, His, and Ile imbalances, there is also an increase in the activity of the major hepatic enzyme regulating the catabolism of the amino acid (Davis and Austic, 1994; Park and Austic, 1998; Torres et al., 1999; Yuan et al., 2000). Growth depressions resulting from dietary amino acid imbalance have been observed for most of the indispensable amino acids of rats and chickens. Phenylalanine, however, is not one of these. The following experiments were performed to create a Phe imbalance and to examine the activity of liver Phe hydroxylase (PAH) under conditions of Phe imbalance or dietary Phe excess.

The objective of experiment 1 was to determine the level of Phe that was needed to meet the requirement of Babcock B380 chicks used in the current study. The objectives of experiment 2 were to create growth depressions from a mixture of amino acids lacking Phe (IAA – Phe) and excess Phe and to determine the activity of PAH under the 2 conditions.

MATERIALS AND METHODS

General

Day-old male Babcock B380 chicks obtained from a commercial hatchery were reared in thermostatically controlled battery cages with raised wire floors and dimensions of 33 × 100 cm. The temperature in the heated section of the cages was set at 32°C, and the room temperature was approximately 21°C during the experiment. Light was provided from 0600 to 2200 h within each 24-h cycle. Chicks were given a preexperimental diet for 1 wk to

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allow them to become accustomed to a semipurified diet. The diet contained (in g/kg of diet) the following ingredients: glucose monohydrate, 663.5; isolated soybean protein, 73.0; isolated soy protein, 182.0; amino acids, 6.6; Phe, 0; Glu, 0; cellulose, 30.0; vitamin premix, 12.0; mineral premix, 65.9; glucose-H2O, 663.5; corn oil, 40.0.

Table 1. Composition of the basal diets in experiments 1 and 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Preexperimental (g/kg of diet)</th>
<th>Basal 1 (g/kg of diet)</th>
<th>Basal 2 (g/kg of diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated soy protein1</td>
<td>182.0</td>
<td>73.0</td>
<td>73.0</td>
</tr>
<tr>
<td>Amino acids2</td>
<td>6.6</td>
<td>63.4</td>
<td>63.4</td>
</tr>
<tr>
<td>Phe</td>
<td>0</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>Glu</td>
<td>0</td>
<td>81.4</td>
<td>79.6</td>
</tr>
<tr>
<td>Cellulose3</td>
<td>30.0</td>
<td>30.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Vitamin premix4</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Mineral premix5</td>
<td>65.9</td>
<td>65.9</td>
<td>65.9</td>
</tr>
<tr>
<td>Glucose-H2O6</td>
<td>663.5</td>
<td>634.3</td>
<td>634.1</td>
</tr>
<tr>
<td>Corn oil7</td>
<td>40.0</td>
<td>40.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

1Composition 93% protein, obtained from Dyets Inc. (Bethlehem, PA).
2Supplied the following (g/kg): amino acids in preexperimental diet: Thr, 1.5; Met, 1.9; Cys, 2.7; Trp, 0.5; amino acids in basal diets: Thr, 4.9; Cys, 3.3; Met, 2.9; Val, 5.2; Ile, 5.4; Leu, 7.8; Tyr, 3.7; His, 2.5; Lys-HCl, 8.1; Arg, 7.9; Trp, 1.5; Gly, 3.9; and Ser, 6.3. All the amino acids were L-isomers.
3Obtained from Teklad Test Diets (Madison, WI).
4Supplied the following in the diet (mg/kg): thiamin HCl, 15.0; riboflavin, 15.0; nicotinic acid, 50.0; p-calcium pantothenate, 20.0; pyridoxine HCl, 6.0; folic acid, 6.0; biotin, 0.6; myo-inositol, 250.0; menadione sodium bisulfite, 1.5; and butylated hydroxytoluene, 100.0. Also supplied premixes of retinyl acetate (650,000 IU/g), 6.9; cholecalciferol (500,000 IU/g), 4.5; α-tocopheryl acetate (500 IU/g), 100; vitamin B12 (0.1% in mannitol), 20; choline chloride (60% choline), 2,850; and glucose monohydrate to make 12 g.
5Supplied the following in the diet (g/kg): CaHPO4, 20.7; CaCO3, 14.8; MgSO4, 3.0; NaHCO3, 3.0; NaCl, 6.0; KH2PO4, 10.0; KHCO3, 6.4; KCl, 1.0; FeSO4·7H2O, 0.5; MnSO4·H2O, 0.35; ZnO, 0.10; CuSO4·5H2O, 0.03; NaMoO4·2H2O, 0.0083; CoCl2·6H2O, 0.0017; KI, 0.0016; and Na2SeO3, 0.0002.
6Obtained from Dyets (Bethlehem, PA).
7Obtained from Goya Foods Inc. (Secaucus, NJ).

The objectives of the second experiment were to create a growth depression from an imbalancing mixture of amino acids lacking Phe (IAA – Phe) and a growth depression arising from excess dietary Phe and to determine the activity of PAH under these conditions. The experiment included 2 treatments in which the imbalance and the toxicity were corrected by the addition of Phe or IAA – Phe, respectively. The 5 dietary treatments were as follows: basal (0.44% Phe), imbalance (basal + 10% IAA – Phe), corrected imbalance (imbalance + 1.12% Phe), excess (basal + 2.00% Phe), and corrected excess (excess + 10% IAA – Phe).

The composition of the added IAA – Phe mixture (in g/kg) was as follows: Thr, 8.6; Tyr, 8.1; His, 5.5; Arg, 17.4; Ile, 12.0; Leu, 17.2; Lys-HCl, 17.9; Met, 2.1; Cys, 1.3; Trp, 2.1; Val, 11.5; NaHCO3, 10.3; and glucose monohydrate, 18.5. In the imbalance, corrected imbalance, and corrected excess treatments, the IAA – Phe mixture was added at the expense of glucose monohydrate.

Each treatment consisted of 5 replicates with 5 chicks per replicate. After 7 d of feeding, the weight gains and feed intakes of each pen of chicks were calculated from initial and final weights of chicks and feed. The chicks were euthanized while in a fed state, and their livers were obtained for analyses of PAH activity.

Tissue Preparation for PAH Assay

Liver samples were collected and prepared by the method of Powell et al. (1999). Two minor modifications made to the method were homogenizing 1 g of liver in 4 mL of KCl solution and using a cutting-dispersing tool instead of a pestle. The homogenizer used was an Ultra-Turrax homogenizer (IKA-Works Inc., Wilmington, NC) with Ultra-Turrax-dispersing tool T25 (S25N 18G). The liver was homogenized at 13,500 rpm for 40 s. The liver
Experiment 1

The weight gain of the chicks increased as Phe increased in the diet from 0.24 to 0.39% in experiment 1 (Table 2). There were no significant differences in the weight gains of the chicks that received the 3 highest levels of dietary Phe. Chicks fed the 2 lowest levels of Phe had significantly lower feed intake than chicks given 0.39% or higher levels of Phe \((P < 0.001)\). Chicks given the 3 highest levels of Phe had significantly higher feed efficiencies than chicks fed the 3 lowest levels of dietary Phe \((P < 0.001)\). The requirement of Phe was estimated to be 0.38% \((0.007 \pm \text{SE})\) of the diet based on weight gain and 0.39% \((0.004 \pm \text{SE})\) of the diet based on feed efficiency.

Experiment 2

The weight gains of the chicks fed the IAA – Phe mixture and excess dietary Phe were significantly lower \((P < 0.05)\) than the weight gains of the chicks fed the basal diet (Table 3). Chicks given excess dietary Phe had the lowest weight gains. The weight gains of the chicks given the diets corrected for the IAA – Phe mixture and excess Phe were equal to and greater than, respectively, the weight gains of chicks fed the basal diet. There were no significant differences in the feed intake of the chicks fed the basal, basal plus the IAA – Phe mixture, or basal plus excess Phe diets. The feed efficiency of chicks given the IAA – Phe mixture was not significantly different from that of the basal group. However, the feed efficiency of the chicks given the excess Phe was significantly lower than that of the chicks given the basal diet. The feed efficiencies of chicks given the corrected diets were not significantly different from those of the chicks given the basal diet.

Although the activity of PAH per milliliter of liver supernatant in chicks given the imbalance diet was not significantly higher than that of the chicks given the basal diet, the PAH activity of chicks given the corrected imbalance diet was significantly higher than that of chicks given the basal diet. The activity of PAH in chicks given the diet containing excess Phe was significantly higher than that of chicks given the basal diet. Chicks given the corrected diet containing excess Phe had significantly higher PAH activity than chicks given the basal diet, but they had lower PAH activities than chicks given the diet containing excess Phe alone \((P < 0.05)\).

In the analysis of the dietary amino acids, the Phe and Tyr contents of isolated soybean protein were 3.56 and 2.97% of DM. The isolated soy protein contained 92.2% DM. The calculated Phe and Tyr contents of basal 1 (as fed) were 0.24 and 0.59%, respectively, and the contents of basal 2 (as fed) were 0.44 and 0.59%, respectively. These values were corrected for 94 and 84% recoveries, respectively, of Phe and Tyr.

DISCUSSION

There is little empirical data on the Phe requirements of light breeds of chickens. Based on published research, the NRC estimated that the Phe requirement of Leghorn-type chicks from 0 to 6 wk of age was 0.54% of the diet, and the requirement for Phe and Tyr combined was 1.00% of the diet (NRC, 1994). These estimates seemed to be based on the studies of Fisher (1956), Fisher et al. (1957), Klain et al. (1960), and Dean and Scott (1965). Fisher et al. (1957) determined that the Phe requirement of New Hampshire male × Columbian crossbred male chicks from 4 to 10 d of age was approximately 0.46% of the diet. The Tyr requirement was estimated to be not more than 0.50% of the diet. Earlier, Fisher (1956) had estimated the Phe requirement to be 0.48% of the diet. Results of the studies by Klain et al. (1960) using male chicks of a similar cross to Fisher et al. (1957) indicated that the Phe requirement...
Table 2. The effects of graded levels of Phe on weight gain, feed intake, and feed efficiency (experiment 1)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Phe (% of diet)</th>
<th>Weight gain (g/chick per d)</th>
<th>Feed intake (g/chick per d)</th>
<th>Feed efficiency (weight gain/ feed intake)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.24</td>
<td>2.8&lt;sup&gt;D&lt;/sup&gt;</td>
<td>9.9&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.29&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.29</td>
<td>4.6&lt;sup&gt;C&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.39&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.34</td>
<td>6.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>14.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.45</td>
</tr>
<tr>
<td>4</td>
<td>0.39</td>
<td>7.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>14.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.53</td>
</tr>
<tr>
<td>5</td>
<td>0.44</td>
<td>7.8&lt;sup&gt;A&lt;/sup&gt;</td>
<td>14.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>0.49</td>
<td>8.4&lt;sup&gt;A&lt;/sup&gt;</td>
<td>14.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.57&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td>0.22</td>
<td>0.44</td>
<td>0.020</td>
</tr>
</tbody>
</table>

<sup>A–D</sup>Means within a column with different superscripts differ significantly (P ≤ 0.001).

***P ≤ 0.001.

Table 3. Weight gain, feed intake, feed efficiency and Phe hydroxylase (PAH) activity of chicks subjected to an imbalancing mixture of amino acids and excess Phe (experiment 2)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Phe (% of diet)</th>
<th>IAA – Phe&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Weight gain (g/chick per d)</th>
<th>Feed intake (g/chick per d)</th>
<th>Feed efficiency (weight gain/ feed intake)</th>
<th>PAH activity&lt;sup&gt;2&lt;/sup&gt; (nmol of Tyr/mL of liver supernatant per 20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0.44</td>
<td>—</td>
<td>8.6&lt;sup&gt;B&lt;/sup&gt;</td>
<td>17.7</td>
<td>0.49&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>275&lt;sup&gt;F&lt;/sup&gt;</td>
</tr>
<tr>
<td>Imbalance</td>
<td>0.44</td>
<td>13.2</td>
<td>7.3&lt;sup&gt;C&lt;/sup&gt;</td>
<td>16.6</td>
<td>0.43&lt;sup&gt;B&lt;/sup&gt;</td>
<td>42&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corrected imbalance</td>
<td>1.56</td>
<td>13.2</td>
<td>9.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>17.4</td>
<td>0.56&lt;sup&gt;A&lt;/sup&gt;</td>
<td>522&lt;sup&gt;BC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Excess</td>
<td>2.44</td>
<td>—</td>
<td>5.4&lt;sup&gt;D&lt;/sup&gt;</td>
<td>15.8</td>
<td>0.34&lt;sup&gt;C&lt;/sup&gt;</td>
<td>1,056&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>Corrected excess</td>
<td>2.44</td>
<td>13.2</td>
<td>9.0&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>17.7</td>
<td>0.51&lt;sup&gt;A&lt;/sup&gt;</td>
<td>667&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td></td>
<td></td>
<td>0.52</td>
<td>1.19</td>
<td>0.035</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<sup>A–D</sup>Means within a column with different superscripts differ significantly (P ≤ 0.001).

1Indispensable amino acid mixture lacking Phe.

2Due to the heterogeneity of variances among the various treatments, the means were transformed using the natural logarithm of the PAH activity. The P-value and SEM are for the transformed data.

3No significant difference in means.

***P ≤ 0.001.
factor may be increased catabolism of Phe by its rate-limiting enzyme, as suggested by Davis and Austic (1982, 1994) in explaining the possible role of Thr catabolism in Thr imbalance.

The negative effect of excess Phe on chickens is well documented (Tamimie and Pscheidt, 1966; Edmonds and Baker, 1987; Keene and Austic, 2001). The depression in growth arising from excess dietary Phe may be attributed to various factors. The concentration of serotonin, for example, was depressed, and the concentration of dopamine in the brain was markedly increased in rats given a large dietary excess of Phe (Green et al., 1962). Depressions in brain serotonin concentrations have been consistently reported in phenylketonuria and rat models of this disease (Brass et al., 1982), and evidence of reduced serotonin in chickens subjected to a large dietary excess of Phe has been reported (Tamimie, 1966a). It is conceivable, therefore, that changes in neurotransmitter concentrations could result in physiological modifications that affect growth. Depression in growth rate could be a consequence of reduced feed consumption. Large excesses of Phe have resulted in reduced feed intake in various species including chickens (Harper et al., 1970). Tamimie (1966b) limited the intake of a practical diet for chicks to the level of intake (40%) that was previously observed in chicks fed the diet containing 2% added Phe (Tamimie and Pscheidt, 1966). The restricted intake resulted in BW and the weights of several internal organs, except liver, that were similar to those of chicks that received the added Phe. Tamimie (1967) observed more severe depressions of feed intake and growth of chicks when 5%, instead of 2%, Phe was added to the practical diet. Edmonds and Baker (1987) reported that the addition of 4% Phe to a practical diet for chicks caused 40 to 48% reductions in feed intake. Keene and Austic (2001) observed 11 and 22%, respectively, lower feed intake in Leghorn chicks that received 1.5 or 2% additions of Phe in a semipurified diet. The feed intakes of the chicks that received 2% added Phe, however, were not depressed in the present experiment. This may have been primarily due to the lower level of Phe in basal 2 than was present in the basal diets of other studies or to the higher amounts of Phe added to create the excesses in the studies of Tamimie (1967) and Edmonds and Baker (1987). It is not clear why the results of experiment 2 differ from that of Keene and Austic (2001), but it should be noted that the breed of chick, the experimental diet, and the length of the experiment were different from those of the present study. The results of experiment 2 suggest that an effect of Phe on food intake is not an obligatory requisite for the depression in growth of chicks fed excess Phe.

The lack of change in PAH activity in the chicks fed the diet containing the 10% IAA – Phe mixture was different from previously reported cases of amino acid imbalances involving Thr, Ile, and His, in which the activities of Thr dehydrogenase, branched-chain ketoacid dehydrogenase, and histidase, respectively, were increased (Davis and Austic, 1982, 1994; Park and Austic, 1998; Torres et al., 1999; Yuan et al., 2000). However, the possibility that PAH activity was increased in chicks fed the imbalance diet cannot be fully ruled out. The in vivo regulation of the mammalian enzyme, for example, involves activation by Phe (Tourian, 1971; Shiman and Gray, 1980; Tipper and Kaufman, 1992; Davies et al., 1997) and level of phosphorylation at Ser16 (Kobe et al., 1999). Phosphorylation results in modifications to structural and allosteric components of PAH as well as an increase in availability of the enzyme to its substrate (Tipper and Kaufman, 1992; Kobe et al., 1999; Johnson and Lewis, 2001; Miranda et al., 2002). Rat PAH was progressively activated as Phe concentrations increased in the assay medium over 7 concentrations in the physiological range from 0 to 1.0 mM in the studies of Shiman et al. (1982). Phosphorylation of PAH makes the enzyme more sensitive to activation by Phe (Shiman et al., 1982). If this is also true for chick PAH, then a change in degree of phosphorylation could result in increased activity in vivo at low liver Phe levels such as could be expected with chicks fed the basal diet or the imbalance diet. High levels of protein (or amino acids) could be expected to increase glucagon secretion (Eisenstein et al., 1979) and, consequently, cyclic adenosine monophosphate-mediated phosphorylation of PAH at Ser16 (Donlon and Kaufman, 1978; Beirne et al., 1985). Increased PAH activity might not have been reflected in the in vitro assay, because, based on the nature of mammalian PAH (Shiman et al., 1982), the assay medium contained more than enough Phe (50 mM) to activate the enzyme.

It should be noted that the mean PAH values for the high-Phe treatments in experiment 2 inflated the SEM values for the statistical analysis. If the basal, imbalance, and imbalance-corrected diets only were included in the ANOVA, the PAH response to the imbalance diet is significant. Because the initial plan was to compare all treatments in a single ANOVA, it was deemed inappropriate to reanalyze the data in a different manner. Nonetheless, one has to consider that there was at least a trend for increased PAH activity in the imbalance group as compared with the basal group.

Excess dietary Phe increased the activity of PAH. These results were in contrast to those reported by Freedland et al. (1964), McCormick et al. (1965), and Schott et al. (1986), who indicated that the activity of hepatic PAH decreased in response to excess Phe in rats. The results also differed from those of Keene and Austic (2001), who reported that the addition of 1 or 2% Phe to a diet of Leghorn chicks did not significantly affect PAH activity. Their basal diet was similar in composition to the present diet except that the diet contained 18.2% isolated soybean protein (93% CP) and small amounts of Cys, Met, Thr, and Trp to ensure that amino acid requirements were met. The basal diet also contained a higher concentration of Phe (76%, not corrected for analytical recovery). There were differences in the amino acid content of the diet, breed of chickens, and method of PAH analysis. It is not clear which, if any, of these differences account for the conflicting results.
The reduction in PAH activity after the addition of the IAA-Phe mixture to the diet containing excess Phe may have been due to an increase in protein synthesis. Amino acids, especially Leu, increase the rate of signal transduction in pathways such as the mammalian target of rapamycin pathway. The stimulation of this pathway then results in increased initiation of mRNA translation and, consequently, an increase in protein synthesis (Vary and Lynch, 2007). Phenylalanine would then be sequestered for the protein synthesis and would not available to stimulate PAH. A second reason for the reduction in PAH activity when the IAA – Phe mixture is added to the diet containing the excess Phe may have been a reduction in reabsorption of Phe from the renal tubules of the kidney due to increased competition between Phe and the additional amino acids for particular transport systems. Urinary losses of Phe might have contributed to less activation of PAH.

ACKNOWLEDGMENTS

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REFERENCES


