Histidine-Imbalanced Diets Stimulate Hepatic Histidase Gene Expression in Rats

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ABSTRACT A high protein concentration in the diet induces the gene expression of several amino acid degrading enzymes such as histidase (Hal) in rats. It is important to understand whether the amino acid pattern of the dietary protein affects the gene expression of these enzymes. The purpose of the present work was to study the effect of a histidine-imbalance diet on the activity and mRNA concentration of rat hepatic histidase. Seven groups of six rats were fed one of the following diets: 1) 6% casein (basal), 2) 20% casein, 3) 35% casein, 4) an imbalance diet containing 6% casein plus a mixture of indispensable amino acids (IAA) equivalent to a 20% casein diet without histidine (I-20), 5) 6% casein plus a mixture of IAA equivalent to a 35% casein diet without histidine (I-35), 6) a corrected diet containing 6% casein plus IAA including histidine equivalent to a 20% casein diet, 7) a corrected diet containing 6% casein plus IAA including histidine equivalent to a 35% casein diet. Serum histidine concentration was inversely proportional to the protein content of the diet, and it was significantly higher in rats fed the corrected diets compared to their respective imbalanced diet groups. Hal activity increased as the protein content of the diet increased. Greater histidine imbalance resulted in lower food intake and higher Hal activity. Rats fed histidine-corrected diets had lower activity than their respective imbalanced groups. Differences in Hal activity were associated with differences in the concentration of Hal mRNA. These results indicate that rats fed a histidine-imbalance diet exhibit reduced food intake and weight gain and increased Hal gene expression as a consequence of an increased amino acid catabolism. J. Nutr. 129: 1979–1983, 1999.

KEYWORDS: • amino acid imbalance • gene expression • histidase • histidine • rats

Histidase (histidine ammonio-lyase EC 4.3.1.3; Hal) is the first enzyme in the catabolism of histidine, catalyzing the deamination of L-histidine to trans-urocanic acid. The expression of the gene encoding this enzyme is induced by dietary protein (Torres et al. 1998) and by glucagon and hydrocortisone (Aleman 1998). This response to the protein content occurs in well nourished, and during nutritional rehabilitation of previously undernourished rats (Tovar et al. 1998). However, the induction of the Hal gene is not dependent on histidine concentration of the diet (Torres et al. 1998).

There is evidence that the intake of imbalanced diets for several days stimulates the activity of some amino acid degrading enzymes. Serine dehydratase activity increases in rats fed a histidine-imbalance diet (Anderson et al. 1969). Also, threonine imbalance increases the activity of threonine dehydrogenase (Davis and Austic 1994). The stimulation of enzyme activity observed in these studies is one of several adaptive mechanisms responding to consuming an imbalanced diet (Gietzen 1993).

On the other hand, the consumption of a diet containing one amino acid below its requirement increases the rate of oxidation for other amino acids (Benevenga et al. 1993). Thus, feeding growing pigs a diet limited in histidine produces an increase in phenylalanine oxidation, and when histidine is added at its required level, phenylalanine oxidation reduces to basal levels (Kim et al. 1983). Studies with rats also showed that consumption of a diet containing levels of threonine below that required increases the catabolism of valine and methionine (Stipanuk and Benevenga 1977). Feeding rats with a diet containing zein as protein source, which essentially lacks lysine and tryptophan, produces an increase in the rate of threonine and leucine oxidation (Kim et al. 1996).

Thus, if the concentration of one indispensable amino acid is below that required, it seems to increase the activity of the amino acid degrading enzymes, however there are no studies at the present time that indicate that this condition results in an induction of the gene expression of the amino acid degrading enzymes. The purpose of the present study was to assess whether feeding highly histidine-imbalance diets affects the activity and mRNA concentration of hepatic histidase, extend the understanding of the effect of excess dietary protein on the stimulation of Hal gene induction, and to gain knowledge about whether disproportion in the amino acid profile of the diet, which is present in low-quality proteins, can modify the expression of the amino acid degrading enzymes, such as Hal.

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Basal and imbalanced diets is described in 0.2% L-methionine and 0.4% L-threonine to improve the nutritional quality of casein. The 20 and 35% casein diets contained 200 and 350 g vitamin-free casein/kg diet, respectively. The concentration of the rest of the ingredients was described previously (Torres et al. 1998). The vitamin-free casein and the rest of the ingredients were obtained from Teklad, Madison, WI.

### MATERIALS AND METHODS

**Reagents and chemicals.** L-Leucine, L-arginine, L-valine, L-phenylalanine, L-isoleucine and L-histidine were a gift from Kyowa Hakko Kogyo, Japan, and L-tryptophan, L-threonine, L-lysine and L-methionine were from Fermentaciones Mexicanas S.A de C.V, México. Nylon membrane filters (Hybond-N+) were purchased from Amersham (Buckinghamshire, UK), and the Gene Clean II kit was purchased from Bio 101 (La Jolla, CA).

**Animals.** Male Wistar rats, obtained from the Experimental Research Department and Animal Care Facilities at the National Institute of Nutrition, México D. F., were housed individually in wire, stainless steel cages at 22°C with a 12-h light-dark cycle and with free access to water.

**Dietary treatments.** Forty-two rats, weighing 75–90 g, had free access to the appropriate diet for 10 d and were randomly divided into seven groups of six rats each: 1) fed 6% casein (basal diet), 2) fed a 20% casein diet (control-20), 3) fed a 5% casein diet (control-35), 4) fed a histidine-imbalanced diet containing 6% casein plus a mixture of indispensable amino acids (IAA) equivalent to a 20% casein diet devoid of histidine (I-20), 5) fed a corrected diet containing 6% casein plus a mixture of IAA including histidine equivalent to a 20% casein diet (Cr-20), 6) fed a histidine-imbalance diet containing 6% casein plus a mixture of IAA equivalent to a 35% casein diet devoid of histidine (I-35), 7) fed 6% casein plus a mixture of IAA including histidine equivalent to a 35% casein diet (Cr-35). At the end of the 10-d period, the rats were anesthetized with carbon dioxide and killed by decapitation between 0900 and 1000 h. Blood was collected and kept at 22°C until amino acid analysis. The liver was dissected immediately, and a tissue sample was frozen in liquid nitrogen for RNA extraction and the rest was used to measure enzyme histidase activity.

**Northern blot analysis.** Total RNA was isolated from the liver according to the method of Chomczynski and Sacchi (1987). For Northern analysis, 20 μg RNA was electrophoresed in a 0.8% agarose gel containing 37% formaldehyde, transferred to a nylon membrane (Hybond-N+) and cross-linked with a UV crosslinker (Amerham). The cDNA probe was a 1.95 kb polymerase chain reaction (PCR) product amplified from rat liver histidase cDNA that was kindly provided by Dr. R. R. McInnes (The Hospital for Sick Children, Toronto, Canada; Taylor et al. 1990). The forward and reverse primers used for the PCR reaction were 5’-ATGCCATGTTA-CACGGTGTCG3’ and 5’-TTAAGATCGTCAGACTCTGTG3’, respectively. The PCR product was purified with Gene Clean and labeled with Redivue [α-32P] deoxyctydine 5’[α-32P] triphosphate (110 TBq/mmol) by using the Rediprime DNA labeling kit. Membranes were prehybridized with rapid-hyb buffer (Amerham) at 65°C for 30 min and then hybridized with the cDNA probe (53.3 MBq/L) for 2.5 h at 65°C. Membranes were washed once with 2X citrate saline solution (SSC) (1X SSC = 0.15 mol sodium chloride/L·15 mol sodium citrate/L)·0.1% SDS at room temperature for 20 min and then twice for 15 min with 0.1X SSC/0.1% SDS at 65°C. Digitized images and quantification of radioactivity (dpm) of the bands were done by using the Instant Imager (Packard Instrument,

### TABLE 1

Composition of the experimental diets

<table>
<thead>
<tr>
<th></th>
<th>Basal diet</th>
<th>20% casein</th>
<th>35% casein</th>
<th>His-imbalance diet-20 (I-20)</th>
<th>His-corrected diet-20 (Cr-20)</th>
<th>His-imbalance diet-35 (I-35)</th>
<th>His-corrected diet-35 (Cr-35)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Casein</strong></td>
<td>60.0</td>
<td>200.0</td>
<td>350.0</td>
<td>60.0</td>
<td>60.0</td>
<td>60.0</td>
<td>60.0</td>
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<tr>
<td><strong>Cornstarch</strong></td>
<td>412.0</td>
<td>345.0</td>
<td>270.0</td>
<td>386.3</td>
<td>384.6</td>
<td>355.5</td>
<td>351.9</td>
</tr>
<tr>
<td><strong>Cellulose</strong></td>
<td>412.0</td>
<td>345.0</td>
<td>270.0</td>
<td>386.3</td>
<td>384.6</td>
<td>355.5</td>
<td>351.9</td>
</tr>
<tr>
<td><strong>Corn oil</strong></td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td><strong>Mineral mix</strong></td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td><strong>Vitamin mixture</strong></td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>L-Methionine</strong></td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>3.5</td>
<td>3.5</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td><strong>L-Threonine</strong></td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>5.3</td>
<td>5.3</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td><strong>L-Arginine</strong></td>
<td>4.6</td>
<td>4.6</td>
<td>4.6</td>
<td>3.5</td>
<td>3.5</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td><strong>L-Histidine</strong></td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
<tr>
<td><strong>L-Isoleucine</strong></td>
<td>11.2</td>
<td>11.2</td>
<td>11.2</td>
<td>23.2</td>
<td>23.2</td>
<td>23.2</td>
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<tr>
<td><strong>L-Leucine</strong></td>
<td>9.8</td>
<td>9.8</td>
<td>9.8</td>
<td>20.3</td>
<td>20.3</td>
<td>20.3</td>
<td>20.3</td>
</tr>
<tr>
<td><strong>L-Lysine</strong></td>
<td>6.2</td>
<td>6.2</td>
<td>6.2</td>
<td>12.8</td>
<td>12.8</td>
<td>12.8</td>
<td>12.8</td>
</tr>
<tr>
<td><strong>L-Phenylalanine</strong></td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>L-Tryptophan</strong></td>
<td>8.4</td>
<td>8.4</td>
<td>8.4</td>
<td>17.4</td>
<td>17.4</td>
<td>17.4</td>
<td>17.4</td>
</tr>
<tr>
<td><strong>L-Valine</strong></td>
<td>5.3</td>
<td>5.3</td>
<td>5.3</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

1 Torres, et al. 1998.

**Determination of histidine concentration.** Pooled samples of serum were prepared by mixing equal amounts of serum from all animals of each dietary treatment group. Protein-free sulfosalicylic acid extracts were prepared as described previously (Tovar et al. 1996) and were analyzed for free amino acid content in a Beckman System 6300 high performance amino acid analyzer (Beckman Instruments, Palo Alto, CA).

**Histidase activity.** One g of liver was washed with ice-cold saline, blotted and homogenized with a polytron (PT2000 Kinematica, Lucerne, Switzerland) at the lowest setting in 4 mL of an ice-cold solution containing 5 mmol NaOH/L in 14 mmol KCI/L. The homogenates were centrifuged for 60 min at 105,000 × g, and the clear supernatant was stored at −80°C before measuring histidase activity. The activity was assayed as described previously (Spolter and Baldridge 1963). The method is based on the spectrophotometric measurement of the appearance of urocanic acid at 277 nm. The reaction was linear for 10 min at 25°C in 0.1 mol pyrophosphate buffer/L, pH 9.2. An enzyme unit was defined as the formation of 1 nmol of urocanic acid/min. The protein concentration was measured by Biuret assay with bovine serum albumin standards.

**Northern blot analysis.** Total RNA was isolated from the liver according to the method of Chomczynski and Sacchi (1987). For Northern analysis, 20 μg RNA was electrophoresed in a 0.8% agarose gel containing 37% formaldehyde, transferred to a nylon membrane (Hybond-N+) and cross-linked with a UV crosslinker (Amerham). The cDNA probe was a 1.95 kb polymerase chain reaction (PCR) product amplified from rat liver histidase cDNA that was kindly provided by Dr. R. R. McInnes (The Hospital for Sick Children, Toronto, Canada; Taylor et al. 1990). The forward and reverse primers used for the PCR reaction were 5’-ATGCCATGTTAACCGGTTCG3’ and 5’-TTAAGATCGTCAGACTCTGTCG3’, respectively. The PCR product was purified with Gene Clean and labeled with Redivue [α-32P] deoxyctydine 5’[α-32P] triphosphate (110 TBq/mmol) by using the Rediprime DNA labeling kit. Membranes were prehybridized with rapid-hyb buffer (Amerham) at 65°C for 30 min and then hybridized with the cDNA probe (53.3 MBq/L) for 2.5 h at 65°C. Membranes were washed once with 2X citrate saline solution (SSC) (1X SSC = 0.15 mol sodium chloride/L·15 mol sodium citrate/L)·0.1% SDS at room temperature for 20 min and then twice for 15 min with 0.1X SSC/0.1% SDS at 65°C. Digitized images and quantification of radioactivity (dpm) of the bands were done by using the Instant Imager (Packard Instrument, Palo Alto, CA).
Meriden, CT). Membranes were also exposed to Extascan film (Kodak) at −70°C with an intensifying screen.

Statistical analysis. Results are presented as mean ± SEM. Statistical analysis was done by one-way ANOVA. Significant differences among groups were determined by Fisher’s protected least square difference test. When the error variance in the groups was heterogeneous, a logarithmic transformation of data was carried out before ANOVA analysis. Differences were considered significant at P < 0.05. The association between serum histidine concentration and protein content of the diet was analyzed by linear regression (Statview statistical analysis program, V.4.5, Abacus Concepts, Berkeley, CA).

RESULTS

Effect of imbalanced diets on body and liver weight and food intake. Liver weight, food intake and histidine consumed were a function of the dietary protein content of the diet (Table 2). These variables were higher in rats fed the 35% than the 6% casein diet. The groups of rats fed imbalanced diets showed lower weight gain and liver weight than the groups fed 6, 20 or 35% casein. Higher amino acid imbalance resulted in lower weight gain in the rats. The histidine-corrected groups showed higher histidine intake, food intake and body weight gain than the groups fed imbalanced diets, but they did not reach the values seen in rats fed the 20 or 35% casein diets. This effect was probably produced by the lack of addition of indispensable amino acids to the corrected diets.

Effect of imbalanced diets on serum histidine concentration. Serum histidine concentration was inversely proportional to the protein content of the diet [r = 0.98, P < 0.05 (Fig. 1)]. Serum histidine concentration in the group fed the I-20 diet was similar to that in rats fed the basal diet, however in rats fed a highly histidine-imbalanced diet (I-35), histidine concentration was lower than in rats fed the basal diet. Histidine concentration in rats fed the corrected diets was higher than their respective imbalanced groups.

Effect of imbalanced diets on Hal activity. Hal activity increased as the protein content in the diet increased. Hal activity in the I-20 and I-35 groups was 2- and 3.2-fold higher than in the basal group [6% (P < 0.01)], and it was 67 and 40% higher than the Cr-20 and Cr-35 groups, respectively (P < 0.01). Greater histidine imbalance increased Hal activity (P < 0.01). Therefore, consumption of a highly histidine-imbalanced diet was associated with a reduction in food intake, leading to an increase in Hal activity (Fig. 2). It would be expected that Hal activity in rats fed the Cr-20 or Cr-35 diets were similar to the groups fed the 20 or 35% casein diets, however Hal activity in the Cr-35 group was lower than in the rats fed the 35% casein diet, which can be explained in part by the lack of addition of dispensable amino acids in the Cr-35 diet.

Effect of imbalanced diets on Hal-mRNA concentrations. Differences in Hal-mRNA concentration followed a pattern similar to that of Hal activity (Fig. 3). Hal-mRNA increased as the concentration of dietary protein increased. Concentrations of Hal-mRNA for the groups fed 20 and 35% casein were 1.1- and 1.9-fold higher than the group fed the 6% casein diet. Rats fed imbalanced diets had lower food consumption than did rats fed the 6% casein diet (Table 2). This condition probably increased protein catabolism, which in turn increased Hal-mRNA concentration and Hal activity. In rats fed the Cr-20 diet, there was a 46% lower abundance of Hal-mRNA compared to the I-20 group, presumably because the increase in food intake reduced protein catabolism. However, in rats fed a Cr-35 diet, there was not a significantly lower concentration of Hal-mRNA with respect to the I-35 group. Rats fed the Cr-35 diet showed a higher Hal-mRNA concentration and Hal activity than did the Cr-20 group.

TABLE 2

Weight gain, liver weight, food and histidine consumption of rats fed histidine imbalanced and corrected diets for 12 d1,2

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain (g/d)</th>
<th>Liver weight (g)</th>
<th>Food consumed (g/d)</th>
<th>Histidine consumed (mg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal (6% casein)</td>
<td>2.6 ± 0.4b</td>
<td>4.9 ± 0.5b</td>
<td>9.8 ± 0.6c</td>
<td>15 ± 1d</td>
</tr>
<tr>
<td>20% casein</td>
<td>7.7 ± 1.0a</td>
<td>7.5 ± 0.3a</td>
<td>17.4 ± 0.7a</td>
<td>87 ± 3b</td>
</tr>
<tr>
<td>35% casein</td>
<td>7.5 ± 3.0a</td>
<td>8.4 ± 0.7a</td>
<td>19.9 ± 0.9a</td>
<td>175 ± 14a</td>
</tr>
<tr>
<td>I-20</td>
<td>2.1 ± 0.3b</td>
<td>3.9 ± 0.3bc</td>
<td>8.1 ± 0.5c</td>
<td>12 ± 2d</td>
</tr>
<tr>
<td>I-35</td>
<td>3.1 ± 0.5b</td>
<td>4.3 ± 0.1b</td>
<td>13.1 ± 0.7b</td>
<td>65 ± 3c</td>
</tr>
<tr>
<td>Cr-20</td>
<td>1.0 ± 0.2c</td>
<td>2.8 ± 0.2c</td>
<td>7.5 ± 0.7c</td>
<td>11 ± 1d</td>
</tr>
<tr>
<td>Cr-35</td>
<td>1.1 ± 0.2c</td>
<td>3.8 ± 0.2bc</td>
<td>10.3 ± 0.9bc</td>
<td>90 ± 8b</td>
</tr>
</tbody>
</table>

1 Results are expressed as means ± SEM, n = 6.
2 Values in a column with different letters are significantly different (P < 0.05). Groups of rats fed 6% casein diet + dispensable amino acids equivalent to 20% (I-20) or 35% casein (I-35) without histidine, 6% casein diet + dispensable amino acids equivalent to 20% (Cr-20) or 35% casein (Cr-35) including histidine.

FIGURE 1 Serum histidine concentration from rats fed 6, 20, or 35% casein diets, highly histidine-imbalanced diets equivalent to 20% (I-20) or 35% (I-35) of indispensable amino acids in casein, and corrected with the addition of histidine equivalent to the amount present in a 20% (Cr-20) or 35% (Cr-35) casein diet, respectively.
weight gain curve plateaus at weight rapidly until the protein requirement is met. The graded levels of dietary protein increase food intake and to both quality and quantity of the dietary protein. Rats fed histidase. can also modify the activity and the mRNA abundance of induction of the Hal gene, but amino acid-imbalanced diets present study that not only do high protein diets stimulate the induced in rats fed high protein diets. We demonstrated in the casein diet, respectively. Values are mean ± SEM, n = 6. Bars with different letters are significantly different (P < 0.05).

DISCUSSION

Previous studies showed that histidase gene expression is induced in rats fed high protein diets. We demonstrated in the present study that not only do high protein diets stimulate the induction of the Hal gene, but amino acid-imbalanced diets can also modify the activity and the mRNA abundance of histidase.

Weight gain and food intake are responses that are sensitive to both quality and quantity of the dietary protein. Rats fed graded levels of dietary protein increase food intake and weight rapidly until the protein requirement is met. The weight gain curve plateaus at ~19% casein (Gustafson et al. 1986). For this reason, rats fed a high protein diet (35%) showed similar weight gain and food intake to rats fed a 20% casein diet.

The effect of imbalanced diets on food intake and growth is well known. Most of the amino acid imbalance studies have used a 6% casein diet as the basal diet, supplementing it with amino acids to reach an equivalent of a 13% casein diet, which provides the theoretical requirement of most amino acids. In the current study we used a more pronounced imbalance, adding to the 6% casein diet a mixture of indispensable amino acids similar to that found in a 20 or 35% casein diet. We observed that the greater the imbalance, the lower final body weight, weight gain, liver weight and food intake (Table 2). Leung and Rogers (1969) demonstrated that the ingestion of imbalanced diets produces a competition among amino acids for entry into the brain that may contribute to the signal for food intake depression.

The depression in growth and food intake in a histidine-imbalanced diet is associated with substantially elevated concentrations of amino acids other than histidine (Harper et al. 1970), and a decrease in the concentration of histidine in serum (Tackman et al. 1990) (Fig. 1) and the brain (Harper 1984). In the blood the high concentration of certain amino acids that share the same amino acid carrier at the blood-brain barrier compete with each other reducing the uptake of the amino acids, present in low concentrations in the plasma, into the brain (Tovar et al. 1988). The altered brain amino acid pattern might initiate, either directly or indirectly, a signal for food intake depression. A decline in the concentration of an indispensable amino acid is sensed in the anterior prepyriform cortex of the brain. Subsequent to recognition of the deficiency, a conditioned anorectic response to ingestion of an amino acid-imbalanced diet develops mediated in part by serotonin at the level of the vagus (Gietzen 1993).

The correction of the 20 or 35% histidine-imbalanced diets by adding histidine partially restored growth and food intake without reaching the weight gain and food intake of the rats with free access to a 20 or 35% casein diets. This probably happened because of the lack of supplementary dispensable amino acids. Under this condition, some proportion of indispensable amino acids is shuttled to the synthesis of dispensable amino acids. Also, because the rate of synthesis of dispensable amino acids from indispensable is relatively slow, this will reduce the efficiency of using all amino acids for protein synthesis, altering the protein accretion rate of the rats (Harper 1983).

The concentration of histidine in serum (Fig. 1) and brain (Gustafson et al. 1986, Peters and Harper 1985) was inversely proportional to the content of casein in the diet. When rats are fed a low-protein diet, serum histidine concentration is high, presumably because the degradation of proteins that contain a high concentration of histidine, especially hemoglobin (Mercer et al. 1989), and to the low activity of histidase (Fig. 2), reducing its catabolism. However, when rats were fed with 35% casein diet, the concentration of histidine in serum...
declined (Fig. 1), and it was associated with an increase in activity and mRNA concentration of Hal (Fig. 3).

When rats were fed a histidine-imbalanced diet, the activity of histidase increased. This increment in Hal activity was accompanied by an increase in Hal-mRNA abundance. Previous studies showed that the activity of some amino acid degrading enzymes, such as serine dehydratase (Anderson et al. 1969) and threonine dehydrogenase (Davis and Austic 1994), increases in animals fed amino acid-imbalanced diets. These results indicate that under amino acid imbalance condition there is an excess of amino acids that is oxidized. The excess of amino acids is probably provided by two pathways: 1) limiting amino acid reduces the efficiency of protein synthesis because all amino acids are required together at the same time; 2) amino acid imbalance reduces food intake, which possibly accelerates the protein turnover, increasing protein catabolism. In support to these mechanisms, it was demonstrated that the oxidation of an indispensable amino acid increases when another amino acid limits protein synthesis (Benevenga et al. 1993, Kim et al. 1983, Stipanuk and Benevenga 1977). Also, the consumption of an amino acid-imbalanced diet diminishes polysome aggregation and incorporation of radiolabeled amino acids into protein especially when the pool of the imbalance amino acid is depleted by the consumption of a diet deficient in this amino acid (Ip and Harper 1974).

The histidine amino acid-imbalanced condition possibly induces Hal by providing the excess of amino acids not used for protein synthesis and by stimulating the secretion of glucagon and glucocorticoids because of the anorexic state. Previous studies (Aleman et al. 1998, Torres et al. 1998, Tovar et al. 1998) support this evidence, although some other still unknown mechanisms may influence the induction of the Hal gene.

The increase in the capacity of the degradation of amino acids by the amino acid degrading enzymes, such as Hal, is perhaps related to the increase in the excretion of urinary nitrogen of rats fed a diet devoid of a single amino acid (Kimura and Tahara 1971). The results of the present study showed that to reach an adequate nitrogen balance, it is important to feed an amino acid-balanced diet, but on the other hand, indicated the need to understand the molecular mechanisms of the regulation of the expression of the amino acid degrading enzymes to conserve the body’s nitrogen.

LITERATURE CITED