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Non-essential amino acids play an important role in adaptation of the rat exocrine pancreas to high nitrogen feeding

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Running title
Adaptation of the exocrine pancreas

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Abstract

We have previously demonstrated that feeding a diet with a high amino acid (60% AA diet) content, as a mixture simulating casein, induced pancreatic growth and pancreatic protease production in rats. In the present study, we examined the effects of an increasing dietary content of essential amino acids (EAA, $x_1 - x_3$ in exp. 1 and $x_1 - x_{3.3}$ in exp. 2) and non-essential amino acids (NEAA, $x_1 - x_3$ in exp. 1 and $x_1 - x_{5.2}$ in exp. 2) on pancreatic growth, amylase and protease adaptation using casein-type amino acid mixtures (exp. 1, basal diet; 20% AA diet) and egg white-type amino acid mixtures (exp. 2, basal diet; 12% AA diet). Pancreatic growth and trypsin activity were induced as the dietary content of NEAA was increased in experiments 1 and 2. Amylase activity in the pancreas was also induced as the dietary content of NEAA was increased, even with the decrease in dietary carbohydrate in experiment 2. The values of all pancreatic variables decreased with the increase in dietary EAA ($x_2$ and $x_3$) without an increase in NEAA. The changes in the pancreas were coincident with increases in plasma arginine and lysine concentrations and a decrease in the plasma alanine concentration. In rats fed a 60% AA diet (EAA and NEAA $x_3$), in the case of which the EAA content was balanced with the NEAA content, pancreatic growth and protease production increased and reached maximum levels as the plasma amino acid concentrations decreased, except for alanine. These results show that NEAA, not EAA, are associated with induction of pancreatic growth and protease production upon feeding a diet with a high AA content, and that some metabolites may be involved in the induction process. The suppression of pancreatic growth and protease production in rats fed the high EAA diets without balanced NEAA may be associated with impairment of amino acid metabolism rather than the increments in the concentration of one or more essential amino acids. Our results also suggest that there is an unknown mechanism or unknown factors involved in regulating pancreatic amylase.
Feeding a high-protein diet induces pancreatic growth and production of pancreatic proteases (1,2). Dietary protein in the lumen of the small intestine competes with endogenous luminal CCK-releasing peptide factors for degradation by pancreatic proteases, resulting in the prolonged survival of the CCK releasing peptides, and this induces pancreatic changes in rats (3-5). We previously demonstrated that feeding a diet containing an amino acid mixture simulating casein also induced pancreatic growth and protease production in rats (6,7). The induction is not mediated by cholecystokinin secretion (6). In humans and dogs, two types of amino acids, phenylalanine and tryptophan stimulate exocrine pancreatic secretion through their action on the intestinal mucosa (8,9). However, we have shown that feeding a diet with a high amino acid content does not induce pancreatic protease production in the lumen of the small intestine using chronic pancreatico-biliary diverted rats (10). Dietary amino acids may stimulate the exocrine pancreas through an unknown mechanism.

The aims of the present study were to determine which kinds of amino acids play a role in the adaptive responses of the exocrine pancreas upon feeding a diet with a high amino acid content. First, we examined the effects of casein-type amino acid mixtures with different ratios of essential and non-essential amino acids (EAA and NEAA) on pancreatic growth, amylase and protease adaptation, because feeding a diet with certain kinds of individual amino acids in excess is known to be toxic in rats (11,12). We used the same amino acid mixture simulating casein as that used in all previous work described above. In experiment 2, we examined the effects of feeding a diet with a high amino acid content on the exocrine pancreas using an amino acid mixture simulating egg white as another type of mixture.

**Experimental Methods**

**Animals and diets**

Male Wistar-ST rats (Japan SLC Inc., Hamamatsu, Japan), weighing about 100 g, were fed diets containing a mixture of amino acids (AA) at a normal level, as shown in Fig. 1 (20% AA diet in exp. 1 and 12% AA diet in exp. 2) (13,14), during the acclimation period. An amino acid mixture simulating casein was used for experiment 1 and a mixture simulating egg white was used for experiment 2, as shown in Table 1.

In experiment 1, the rats were fed a diet containing AA simulating casein with a
normal nitrogen level (20% AA diet) for 7 days and were divided into 6 groups of 6 rats on the basis of body weight. The rats in the 6 groups were given 6 test diets containing various AA for 5 days because we previously observed that pancreatic protease activity reached a maximum level after 5 days of feeding a high-AA diet. The test diets contained AA with various ratios of essential and non-essential amino acids as shown in Fig. 1A. All rats were killed without fast at 10:00 - 11:00 under pentobarbital anesthesia (50 mg/kg body weight; Abbott Laboratories, North Chicago, IL) after sampling of blood from the aorta. The whole pancreas was removed immediately, frozen in liquid nitrogen, and stored at - 40°C until subsequent analyses. We confirmed that the stomach was sufficiently filled with chyme in the case of each rat.

In experiment 2, the rats were fed a diet containing AA simulating egg white with a low nitrogen level (12% AA diet) for 7 days and were divided into 5 groups of 8 rats. In this experiment, we set the AA content at 12% as the lowest, a level supporting maximum growth in rats, and 40% as the highest, a level not suppressing food intake or growth of the rats. The rats in the 5 groups were given the 5 test diets containing various AA shown in Fig. 1B and Table 1. We arranged test diets in group B and C as total amino acid content were approximately double and triple compared with that in group A with fixed NEAA content, and arranged test diets in group C, D and E as EAA content were double and triple with fixed total amino acid content in diet (40%). The rats were killed 7 days after the start of feeding the test diets, as in experiment 1, without sampling of blood from the aorta. Other details were the same as in experiment 1.

This study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Analyses

Trypsinogen in freeze-dried pancreas samples was activated by treatment with enterokinase (Sigma Chemical Co., St. Louis, MO) at 30°C for 20 min in 15 mmole/l Tris buffer (pH 8.1). Pancreatic trypsin activity was measured spectrophotometrically using a synthetic substrate, Nα-p-toluenesulfonyl-L-arginine methyl ester (TAME) (15). Amylase activity in the pancreas was measured with Procion yellow starch (16). Protein was measured by a modified version of Lowry's method (17, 18).
Amino acids in plasma were analyzed by HPLC as phenyl thiocarbamyl (PTC) derivatives with phenyl isothiocyanate (19) after deproteinization with an equal volume of acetonitrile. The HPLC system was equipped with a mini-solvent delivery system, M-600 (Waters), and a Wakopak WS-PTC column (4.0 x 200 mm; Wako Pure Chemical Industries, Osaka, Japan)

Calculations

Trypsin activity was expressed as TAME U/100 g body weight (total activity) in the pancreas. One unit of trypsin activity was defined as the amount of activity resulting in hydrolysis of 1 µmole of substrate per minute at 30°C. Procion yellow starch, the substrate for the amylase assay, was calibrated using a purified α-amylase from porcine pancreas (Type 1A, Sigma Chemical Co.) at 37°C. The data were analyzed by one-way analysis of variance. The existence of a significant difference between groups was determined by Duncan's multiple range test (20) (P < 0.05, SAS version 6.07, SAS Institute Inc., Cary, NC).

Results

Body weight gain and food intake seemed to decrease as a result of feeding a high-nitrogen or high-EAA diet (Table 2). In experiment 1, the values of both parameters were lower in the case of rats fed either of the two test diets containing the highest level of essential AA (diets E and F) than those in the case of rats fed the diet containing 20% AA. In experiment 2, food intake was somewhat lower in the three high-nitrogen groups (diets C, D and E) than in the 12% AA group, however, comparing groups fed diets with the same high AA content, feeding diets containing a higher EAA ratio did not affect food intake. There was no difference in body weight gain among the groups in experiment 2.

Pancreatic dry weight after feeding the diet with the highest NEAA ratio (diet C) was higher than that after feeding the diet containing 20% AA (diet A, Table 3), and a similar tendency was evident in the case of the pancreatic protein content in experiment 1. The protein content of the pancreas was lower in the high EAA ratio groups (diets D and E) than in the other groups.

The amylase content was lower in the groups fed the diets containing a high ratio of EAA (diets D and E) than in the other groups (Fig. 2A). The trypsin (trypsinogen) content of
the pancreas was the highest in the 60% AA group. Increases in the NEAA content of the diet, but not the EAA content, resulted in an increase in pancreatic trypsin activity levels (Fig. 2B). The pancreatic trypsin activities in the case of both groups fed the high EAA ratio diets were lower than those in the other groups.

The changes in plasma amino acid concentrations that occurred as a result of feeding the test diets are shown in Fig. 3. The plasma arginine concentration decreased and the alanine concentration increased gradually with the increasing NEAA content of the diet (diets B and C vs diet A). The arginine concentration was very high and the alanine concentration was low in both of the high EAA ratio groups (diets D and E). The methionine and leucine concentrations were higher as the EAA ratio of the diet increased (diets D and E vs diet A). The concentration of alanine increased and those of other amino acids shown in Fig. 3 decreased as a result of addition of NEAA to the high EAA diet (diet E vs diet F). The serine concentration varied in a manner similar to the alanine concentration. The lysine concentration varied in a manner similar to the arginine concentration, except for a significantly lower value in the case of the 60% AA group as compared to the 20% AA group. Valine and isoleucine concentrations varied in a manner similar to the leucine concentration as a result feeding the test diet containing 20% AA. Other amino acid concentrations did not change markedly (data not shown).

In experiment 2 using AA simulating egg white, increasing the NEAA content resulted in an increase in the values of all pancreatic variables, pancreatic dry weight and protein, levels of amylase and trypsin activity in the pancreas (diets A, B and C in Table 3 and Fig. 4). No marked changes in the values of these variables were observed upon increasing the ratio of EAA, comparing the results for diets with the same AA content (diets C, D and E).

Discussion

We have previously demonstrated that an increase in dietary intake of an amino acid mixture simulating casein induced pancreatic protease production (7). In the present study, we examined the role of essential and non-essential amino acids in the diet on adaptation of the exocrine pancreas using amino acid mixtures composed of essential amino acids (EAA) and non-essential amino acids (NEAA). Pancreatic growth and trypsin were induced with increases in the amounts of NEAA in the diets, in both experiments 1 and 2. In contrast, increases in
EAA resulted in a decrease in the values of all pancreatic variables examined. These results suggest that NEAA, not EAA, play a role in induction of the changes in the exocrine pancreas observed upon feeding a high-AA diet.

In rats fed diet containing high EAA with low NEAA (group D and E in exp. 1), food intake and body weight gain were lower than those on other groups. These lower growth possibly suppressed pancreatic variables shown in the high EAA groups of the present study. However, it is not true in the high EAA and high NEAA group (60% AA in exp. 1). Also, we presented the results of pancreatic variables as values per unit body weight. Moreover, we killed rats in morning and confirmed the stomachs of all rats were filled with sufficient chyme. This eliminates the effects of different feeding patterns among groups on the pancreas, if it were.

Some NEAA are known to affect digestive functions. Glutamine and glutamic acid are important for maintaining the intestinal mucosa (21,22). Changes in intestinal function may affect the exocrine pancreas. However, pancreatic protease production was similarly induced in experiments 1 and 2 in spite of the fact that there was no glutamate in the AA mixture used in experiment 2 (Table 1). Also, the plasma glutamine concentration was not affected as a result of feeding the test diets (data not shown). These amino acids may not be involved in the induction of protease production. Arginine is a potent insulinotropic amino acid (23), and insulin is important to maintain the exocrine pancreas (24). However, the plasma arginine concentration changed in a reciprocal manner, compared to the changes in pancreatic amylase and trypsin activity levels. It seems unlikely that arginine is involved in adaptation of the pancreas upon intake of a high-AA diet. It has been proposed that amino acid sensors are present in the duodenojejunum and the port-hepatic system (25,26). Possibly, amino acids in the diet regulate the exocrine pancreas through such amino acid sensors.

It is recognized that dietary carbohydrates are the inducers of pancreatic amylase production (27,28). A decrease in the carbohydrate content of the diet usually results in a decrease in amylase production. In experiment 2, amylase production was induced by increased ingestion of NEAA even when the carbohydrate content of the diet was reduced from 80% to 50% (from diet A to diet C). In experiment 1, the amylase activity did not increase, but was
maintained at a constant level, with ingestion of increasing amounts of NEAA. The amylase activity in the basal group (20% AA diet) in experiment 1 was high compared with that in the basal group (12% AA diet) in experiment 2. A possible reason for the different results obtained in experiments 1 and 2 is that the basal diet in experiment 1 contained a greater amount of NEAA than that in experiment 2. Amino acids, especially NEAA, in the basal diet may be utilized by rats very efficiently in experiment 2, and the amount of NEAA required for induction of pancreatic amylase may be low. Some surplus NEAA metabolites, for example, glucogenic intermediates, are possibly involved in the induction of pancreatic amylase production. Our findings suggest that there is an unknown mechanism or unknown factors involved in regulating pancreatic amylase. Amylase in the pancreas is also influenced by nutritional status (29,30). Food intake and body weight gain were reduced in the EAAx3 group (diet E in exp.1), but not in the EAAx2 group (diet D) in spite of the fact that the amylase level and the values of other variables were lower in both of the groups. It is not likely that impaired nutritional status caused by lowered food intake is involved in the suppression of amylase production and the function of the exocrine pancreas.

Ingestion of diets with increasing EAA content suppressed pancreatic growth and the levels of pancreatic enzyme activity. There was no evidence of such suppression in the cases where the high EAA was balanced by addition of NEAA (group F in exp. 1, groups D and E in exp. 2). It is reported that intravenous high amino acid loading suppressed exocrine pancreatic secretion (31,32). The plasma arginine concentration shown in Fig. 3 was markedly higher in both high-EAA groups (diets D and E in exp. 1) and the alanine concentration was lower in these groups, but these changes did not show dose dependence. These results suggest that nitrogen metabolism is impaired in rats fed diets containing high EAA content without it being balanced with NEAA. Methionine and leucine concentrations were also higher in the EAAx3 group (diet E), but not in the EAAx2 group (diet D), than in the three EAAx1 groups (diets A, B, and C). The suppression of the exocrine pancreas did not depend on the dietary content of EAA. This finding suggests that the suppression is associated with impairment of amino acid metabolism rather than the increments of concentration of one or more essential amino acids. Results of the present study possibly show that feeding the diet containing high EAA without
balancing NEAA possibly be toxic for the exocrine pancreas.

In rats fed the high-AA diet (the 60% AA group, diet F), in the case of which the EAA content is balanced with the NEAA content, pancreatic growth and pancreatic protease increased and reached maximum levels as the plasma amino acid concentrations decreased, except for alanine. This shows that NEAA improve body amino acid metabolism. In experiments 1 and 2, the increase in pancreatic trypsin activity observed is likely to depend on the total AA content of the diet, except for the EAA-excess diets. The level of protease activity in rats fed diet A in experiment 2 (12% AA) was lower than that in rats fed diet A in experiment 1 (20% AA). The level of activity was similar among the rats fed diets C, D and E in experiment 2, where the amino acid content was the same in spite of the fact that the composition in terms of individual AA was substantially different among these three diets. These findings suggest that the total amount of amino acids is a factor controlling pancreatic protease if the metabolism of amino acids is not impaired. These findings are consistent with the above speculation that some amino acid metabolites induce changes in exocrine pancreatic function.

We have shown in previous studies and in experiment 1 that feeding a high-AA diet containing an AA mixture simulating casein induces pancreatic protease production. In the present study, we showed that ingestion of a diet containing a high level of an AA mixture simulating egg white also induces pancreatic protease production. This result suggests that induction of protease production by a high-AA diet is a common phenomenon, not specific to the AA mixture simulating casein.

In conclusion, pancreatic enzyme production and pancreatic growth were found to be induced by ingestion of diets containing a high level of an AA mixture simulating either casein or egg white. Increases in the levels of non-essential amino acids, but not essential amino acids, in the diet are associated with this induction process, and results of the present study suggest that amino acid metabolites are involved.
References


Table 1  Composition of amino acid (AA) mixtures simulating casein or egg white*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Casein type</th>
<th>Egg white type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g / kg amino acid mixture</td>
<td></td>
</tr>
<tr>
<td>Essential amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L -Histidine-HCl</td>
<td>34.2</td>
<td>23.8</td>
</tr>
<tr>
<td>L -Isoleucine</td>
<td>48.0</td>
<td>53.3</td>
</tr>
<tr>
<td>L -Leucine</td>
<td>88.4</td>
<td>84.6</td>
</tr>
<tr>
<td>L -Lysine-HCl</td>
<td>90.1</td>
<td>65.7</td>
</tr>
<tr>
<td>L -Methionine</td>
<td>25.8</td>
<td>37.1</td>
</tr>
<tr>
<td>L -Phenylalanine</td>
<td>49.7</td>
<td>57.1</td>
</tr>
<tr>
<td>L -Threonine</td>
<td>34.6</td>
<td>42.8</td>
</tr>
<tr>
<td>L -Tryptophan</td>
<td>13.3</td>
<td>15.2</td>
</tr>
<tr>
<td>L -Valine</td>
<td>57.7</td>
<td>69.5</td>
</tr>
<tr>
<td>Non-essential amino acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L -Alanine</td>
<td>28.4</td>
<td>59.0</td>
</tr>
<tr>
<td>L -Arginine-HCl</td>
<td>40.4</td>
<td>56.1</td>
</tr>
<tr>
<td>L -Aspartic acid</td>
<td>25.8</td>
<td>-</td>
</tr>
<tr>
<td>L -Asparagine</td>
<td>33.3</td>
<td>105</td>
</tr>
<tr>
<td>L -Cystine</td>
<td>2.7</td>
<td>29.4</td>
</tr>
<tr>
<td>L -Glutamic acid</td>
<td>89.2</td>
<td>-</td>
</tr>
<tr>
<td>L -Glutamine</td>
<td>103</td>
<td>133</td>
</tr>
<tr>
<td>Glycine</td>
<td>16.4</td>
<td>34.3</td>
</tr>
<tr>
<td>L -Proline</td>
<td>117</td>
<td>34.3</td>
</tr>
<tr>
<td>L -Serine</td>
<td>47.1</td>
<td>61.8</td>
</tr>
<tr>
<td>L -Tyrosine</td>
<td>54.6</td>
<td>38.1</td>
</tr>
</tbody>
</table>

*All amino acids used were crystallized preparations (Ajinomoto Co., Tokyo, Japan). In the case of the amino acid mixture simulating egg white, aspartic acid and glutamic acid were replaced with asparagine and glutamine to prevent acidification of the diet with a high ratio of non-essential amino acids.
Table 2 Body weight gain and food intake of rats fed test diets containing amino acid mixtures simulating casein (exp. 1) or egg white (exp. 2), with various ratios of essential to non-essential amino acids (EAA to NEAA)

<table>
<thead>
<tr>
<th>Diet group</th>
<th>exp. 1</th>
<th>exp. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(EAA) (NEAA)</td>
<td>Body weight gain</td>
</tr>
<tr>
<td>A</td>
<td>(8.8) (11.2)</td>
<td>7.60 ± 0.199 a</td>
</tr>
<tr>
<td>exp.2</td>
<td>(5.4) (6.6)</td>
<td>7.19 ± 0.385 ab</td>
</tr>
<tr>
<td>B</td>
<td>(8.8) (22.4)</td>
<td>7.29 ± 0.453 ab</td>
</tr>
<tr>
<td>exp.2</td>
<td>(5.4) (20.5)</td>
<td>7.42 ± 0.320 a</td>
</tr>
<tr>
<td>C</td>
<td>(8.8) (33.6)</td>
<td>5.98 ± 0.326 b</td>
</tr>
<tr>
<td>exp.2</td>
<td>(5.4) (34.6)</td>
<td>6.15 ± 0.294 b</td>
</tr>
</tbody>
</table>

P values 0.006 <0.001 0.254 0.003

More details on diet groups A, B, C, D, E and F are those shown in Fig. 1. The values shown are mean ± SEM for 6 rats (exp. 1) or 8 rats (exp. 2). Superscript letters are employed to indicate significant differences; values in each column not sharing the same letter are significantly different.

(P <0.05)
Table 3 Pancreatic dry weight and protein content of rats fed test diets containing amino acid mixtures simulating casein (exp. 1) or egg white (exp. 2), with various ratios of essential to non-essential amino acids (EAA to NEAA)

<table>
<thead>
<tr>
<th>Diet group</th>
<th>exp. 1 (EAA)</th>
<th>exp. 1 (NEAA)</th>
<th>Dry weight</th>
<th>Protein content</th>
<th>exp. 2 (EAA)</th>
<th>exp. 2 (NEAA)</th>
<th>Dry weight</th>
<th>Protein content</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>exp.1 (8.8)</td>
<td>111 ± 1.6 bc</td>
<td>87.0 ± 3.41 b</td>
<td>103 ± 4.4 c</td>
<td>68.0 ± 2.77 c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>exp.2 (5.4)</td>
<td>124 ± 4.4 ab</td>
<td>94.3 ± 2.70 ab</td>
<td>106 ± 1.6 bc</td>
<td>71.6 ± 1.79 bc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>exp.1 (8.8)</td>
<td>127 ± 4.2 a</td>
<td>96.8 ± 5.85 ab</td>
<td>115 ± 4.3 ab</td>
<td>77.8 ± 3.42 ab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>exp.2 (5.4)</td>
<td>108 ± 4.6 c</td>
<td>74.1 ± 2.44 c</td>
<td>122 ± 2.3 a</td>
<td>81.9 ± 3.50 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>exp.1 (26.4)</td>
<td>102 ± 6.8 c</td>
<td>71.1 ± 4.52 c</td>
<td>121 ± 3.2 a</td>
<td>84.9 ± 2.80 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>exp.2 (11.2)</td>
<td>129 ± 4.1 a</td>
<td>105 ± 5.0 a</td>
<td>106 ± 1.6 bc</td>
<td>71.6 ± 1.79 bc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>exp.1 (26.4)</td>
<td>124 ± 4.4 ab</td>
<td>94.3 ± 2.70 ab</td>
<td>106 ± 1.6 bc</td>
<td>71.6 ± 1.79 bc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>exp.2 (33.6)</td>
<td>111 ± 1.6 bc</td>
<td>87.0 ± 3.41 b</td>
<td>103 ± 4.4 c</td>
<td>68.0 ± 2.77 c</td>
<td></td>
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</tr>
</tbody>
</table>

More details on diet groups A, B, C, D, E and F are those shown in Fig. 1. The values shown are mean ± SEM for 6 rats (exp. 1) or 8 rats (exp.2). Superscript letters are employed to indicate significant differences; values in each column not sharing the same letter are significantly different.

(P <0.05)
Fig. 1  Diet groups in the present study. Amino acid mixtures simulating casein and egg white were used, as shown in Table 1. Corn oil (50 g/kg diet) containing retinyl palmitate (7.66 µmole/kg diet) and ergocalciferol (0.0504 µmole/kg diet) was used. The mineral mixture was prepared based on that established at the AIN-76 Workshop held in 1989 (Reeves 1989). It provided (mg/kg diet): Ca, 4491; P, 2997; K, 3746; Mg, 375; Fe, 100; I, 0.32; Mn, 10.0; Zn, 34.7; Cu, 6.00; Na, 4279; Cl, 6542; Se, 1.05; Mo, 1.00; Cr, 0.50; B, 0.50; V, 0.25; Sn, 2.00; As, 1.00; Si, 20.0; Ni, 1.00; F, 2.72; Co, 0.20. The vitamin mixture was prepared in accordance with the AIN-76 mixture (AIN 1976) except that menadione and L-ascorbic acid were added to make 5.81 µmole/kg and 284 µmole/kg diet, respectively. In experiment 1, we added NaHCO₃ to neutralize the acidic amino acid mixtures.

Fig. 2  Levels of amylase and trypsin activity in the pancreas after feeding diets containing a mixture of amino acids simulating casein, with various amounts and ratios of essential (EAA) and non-essential (NEAA) amino acids. The P values estimated by one-way analysis of variance were <0.001 in the case of both variables. Mean values not sharing a letter are significantly different between diet groups (P <0.05).

Fig. 3  Amino acid concentration in plasma prepared from aortic blood collected after feeding diets containing an amino acid mixture simulating casein, with various amounts and ratios of essential (EAA) and non-essential (NEAA). P values estimated by one-way analysis of variance were <0.001 in the case of all variables. Mean values not sharing a letter are significantly different between diet groups (P <0.05).

Fig. 4  Levels of amylase and trypsin activity in the pancreas after feeding diets containing an amino acid mixture simulating egg white, with various amounts and ratios of essential (EAA) and non-essential (NEAA) amino acids. P values estimated by one-way analysis of variance were 0.008 for amylase and 0.005 for trypsin. Mean values not sharing a letter are significantly different between diet groups (P <0.05).

Fig. 5  Abundance of cholecystokinin (CCK) and secretin mRNA in the jejunal mucosa after feeding a 60% casein diet or a 60% amino acid (AA) mixture diet compared with a 20% casein diet or a 20% AA mixture diet, for 10 days. Values shown are the mean and standard error for six rats. P values estimated by two-way analysis of variance were 0.020, 0.297 and 0.029 for nitrogen source (S), level (L) and S x L in the case of CCK mRNA, 0.170, 0.790 and 0.356 for nitrogen source, level and S x L in the case of secretin mRNA. Mean values not sharing a letter are significantly different between diet groups (P<0.05).
Fig. 1
Fig. 2

**A**

- Amylase, U/100 g body weight
- % in diet
  - EAA: 8.8 (x1), 11.2 (x1), 20.0 (x1)
  - NEAA: 33.6 (x3), 22.4 (x2)
  - Total AA: 42.4, 42.4

**B**

- Trypsin, TAME U/100 g body weight
- % in diet
  - EAA: 8.8 (x1), 11.2 (x1), 17.6 (x2)
  - NEAA: 33.6 (x3), 22.4 (x2), 11.2 (x1)
  - Total AA: 42.4, 42.4

Legend:
- a
- b
- c
- d

Note: The figures are showing the results of different treatments, with letters indicating statistically significant differences.
Fig. 3